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MODIFICATION OF THE FREQUENCY OF CHROMOSOMAL REARRANGEMENTS INDUCED BY X-RAYS IN DROSOPHILA. III. EFFECT OF SUPPLEMENTARY TREATMENT AT THE TIME OF CHROMOSOME RECOMBINATION

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Received March 7, 1946

THE two preceding papers of this series have described the effects of near infrared radiation (λ ca. 10,000 Å) and of ultraviolet radiation (λ 2,537 Å) in modifying the frequency of chromosomal rearrangements induced by X-ray treatment of the spermatozoa of *Drosophila melanogaster* (KAUFMANN, HOLLAENDER, and GAY 1946; KAUFMANN and HOLLAENDER 1946). In the course of the first series of experiments, females that had been inseminated by X-rayed males deposited eggs during the period of exposure to the near infrared radiation. Larvae developing from these eggs provided material for determining the effects of such exposure on chromosome recombination. In order to compare the effect of temperatures within the range customarily used in raising laboratory cultures of Drosophila, other groups of females inseminated by males receiving identical X-ray treatment were kept at either 18°C or 28°C during the period of fertilization and early cleavage.

Drosophila provides suitable material for measuring the influence of temperature and other conditions on chromosome recombination apart from any effect on the initial production of the breaks by X-rays. According to the interpretation of MULLER (1940) and KAUFMANN (1940, 1941), based on studies of fractionation of the X-ray dose, as well as collateral lines of evidence, the potential breaks induced by irradiation of the mature spermatozoa of the adult male are not utilized in forming new combinations of chromosomes until after the sperm nucleus has penetrated the egg in fertilization. Thus, in the experiments here reported, to measure the effect of temperature on chromosome recombination, several males were treated with a 4,000-roentgen dose of X-rays, mated with virgin females of the same stock (Oregon-R), and kept at 18°C for about 36 hours. The males were then discarded, and the inseminated females divided into three groups, one of which was put into an 18-degree incubator, another into a 28-degree incubator, and the third exposed to near infrared radiation during the period of oviposition. Subsequently the eggs were transferred to the 18-degree cold room for completion of the embryonic and larval development. Eggs were collected at approximately 12-hour intervals; no effort was made to handle them individually so as to determine accurately the termination of the period of syngamy and early cleavage during which chromosome recombination is presumably effected. Thus, varying proportions of the earlier embryonic development in individuals of two of the three groups were completed either at 28 degrees or while exposed to the near infrared radiation. Determination of the fre-

quency of chromosomal rearrangements and the number of breaks involved was made by a cytological analysis of the salivary glands of the third-instar female larvae.

MULLER and PONTECORVO (MULLER 1940) found that temperatures as different as 13°C, 24°C, and 29°C at the time of fertilization produced no significant differences in the frequencies of X-ray-induced translocations between the second and third chromosomes of *D. melanogaster*. Analysis by genetic methods readily permitted detection of translocations between these two chromosomes, but furnished no indication of the complexity of the rearrangements. The cytological method used in the present study offers an opportunity for the detection of breaks in all the chromosomes, except those breaks restricted to heterochromatin, so that intrachromosomal rearrangements as well as interchromosomal exchanges may be considered.

RESULTS

Analysis of 906 pairs of glands provided the data presented in table 1. The group of eggs exposed to the near infrared radiation provided 37.50±2.98

TABLE 1

Frequency of chromosomal rearrangements induced by 4,000 r of X-rays.

TEMPERATURE DURING FERTILIZATION	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING CHANGES	NO. OF BREAKS OBSERVED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
18°C	329	232	97	29.48±2.51	238	72.34	2.45
28°C	313	216	97	30.99±2.61	253	80.83	2.61
Near infrared	264	165	99	37.50±2.98	266	100.76	2.69

percent of rearrangements, a value that differs significantly from the 29.48 ±2.51 obtained in the 18-degree sample ($\chi^2=4.253$; N=1; P=ca. .036). Differences were not significant between the 18- and 28-degree samples, or between the latter and the near infrared group, with respect to the frequency of rearrangements. A further test, using the χ^2 method, was made by comparing the frequency of rearrangements in the near infrared material with that obtained in a group of about 1,800 pairs of glands from a series of experiments in which temperatures of 18°C or 28°C were maintained during syngamy and early cleavage stages. The results, presented in table 2, indicate that the probability is low (P=ca. .02) that the differences are due entirely to sampling errors. It appears, therefore, that near infrared radiation, applied at the time that chromosome recombination presumably is effected, increases the frequency of rearrangements that are detectable by analysis of salivary-gland cells.

The increased frequency of rearrangement is not attributable to selective recombination of specific chromosome regions. The breaks are distributed among the chromosomes approximately in proportion to their lengths, and the χ^2 test indicates that the probability is high (P = ca. .72) that differences in distribution among the 18-degree, 28-degree, and near infrared groups may be attributed to errors of sampling.

DISCUSSION

The effect of near infrared radiation applied during the period of oviposition in increasing the frequency of X-ray-induced chromosomal rearrangements contrasts with that produced by temperatures within the range of 18 to 28°C. The efforts of MULLER and PONTECORVO to modify the frequency of chromosome recombination at the time of fertilization were carried out within the

TABLE 2

Frequency of chromosomal rearrangements induced by 4,000 r of X-rays. Comparison of effect of near infrared radiation during syngamy and e: ly cleavages (264 pairs of glands) with effect of temperatures in the range from 18 to 28°C (1,842 pairs of glands).

	DISTRIBUTION AMONG SPERMS TESTED		
	WITHOUT REARRANGEMENT	WITH REARRANGEMENT	TOTAL
Observed	165	99	264
Expected	(181.33)	(82.76)	
Observed	1,281	561	1,842
Expected	(1,264.67)	(577.24)	
	$\chi^2 = 5.326$ N = 1 P = ca. .02		

temperature range customarily used for raising cultures of *D. melanogaster*. Most of the previous experiments designed to measure the effect of temperature on X-ray-induced breaks in Drosophila have been concerned with the period during which the X-ray treatment was administered, in order to determine whether the ionization initiated chemical reactions that were modifiable by temperature, or whether there was a more direct effect on the chromonemata. The earlier studies of PAPALASHWILI (1935) and MICKEY (1939), which indicated that low temperatures increase the frequency of chromosomal rearrangements, were not confirmed by the extensive work of MULLER and MAKHIJANI (MULLER 1940) on translocations between the second and third chromosomes and on sex-linked lethal mutations, which are known to include some chromosomal rearrangements. The latter finding, also reported earlier by MULLER (1930) has been confirmed by TIMOFÉEFF-RESSOVSKY and ZIMMER (1939). In addition, PLOUGH and EHRENFELD (PLOUGH 1941) found that the production of translocations is not appreciably influenced by temperature, or by temperature shocks (when larvae are treated).

The more extensive data provided by studies on plant chromosomes are complicated by the fact that in such a genus as Tradescantia breakage and

recombination occur concurrently. Lower temperatures (ca. 3°C) at the time of irradiation generally result in increased frequency of aberrations as compared with higher temperature (ca. 33°C), probably by delaying restitution so that the broken ends are available for establishing rearrangements (SAX and ENZMANN 1939; FABERGÉ 1940; RICK 1940), although SAX and ENZMANN report that a reversed effect of temperature occurs when the chromosomes of Tradescantia are irradiated during the earliest prophase stages.

Near infrared radiation also effects an increase in the frequency of chromosomal rearrangement when used prior to X-rays. Various possible modes of action have been considered in the first publication of this series (KAUFMANN, HOLLAENDER, and GAY 1946). The amount of energy available (ca. 1.2 electron volts for λ 10,000 Å) is perhaps sufficient to induce molecular rotation and to initiate low-grade chemical reactions. Because of these properties, the effective action of radiation of this type is probably not limited to an extension of the range of temperature within which fertility and viability may be maintained in Drosophila, but may depend on selective action on certain cellular components (KAUFMANN, HOLLAENDER, and GAY 1946). For a more specific definition of these properties, an extension of our knowledge of infrared absorption spectra in biological materials will be required, as well as studies of the effects of various other wave length regions of the infrared. In more general terms, any cellular changes induced by absorption of near infrared radiation that facilitate chromosome movement at the time that the potential breaks become available to participate in recombination will provide the physical basis for increased frequency of recombination as compared with that of untreated material.

SUMMARY

Eggs deposited by females of *Drosophila melanogaster* that had been inseminated by males previously exposed to 4,000 roentgens of X-rays were kept either at 18°C or at 28°C, or exposed to near infrared radiation (λ ca. 10,000 Å) during the period of syngamy and early cleavage in order to measure the effect of these agents on chromosome recombination.

The frequency of chromosomal rearrangements detected by analysis of salivary gland chromosomes was higher in the group exposed to the near infrared radiation than in the groups kept at 18 or 28°C (table 1). The χ^2 test indicates that the values obtained following the use of near infrared are significantly higher than in the large group of about 1,800 pairs of glands secured in a series of experiments in which temperatures of 18°C or 28°C were maintained during syngamy and early cleavage stages.

Although it increases the temperature within the organism during the period of exposure, the effective action of near infrared radiation in facilitating recombination among the breaks induced by the ionizing radiation is probably not limited to an extension of the range of temperature within which fertility and viability may be maintained in Drosophila, but may depend on selective action on certain cellular components.

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ON THE EFFECTS OF THE GENE *a* ON THE CHEMICAL COMPOSITION OF EPHESTIA KÜHNIELLA ZELLER

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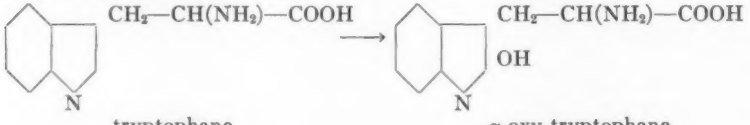
Received March 21, 1946

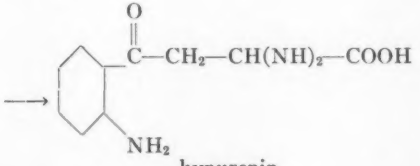
INTRODUCTION

THE gene *a* in the flour moth *Ephestia kühniella* is an eye color mutant—that is, its most obvious phenotypic effect consists in a reduction of the size and number of the pigment granules in the eyes. Pigmentation in other parts of the body is also reduced. Color mutants constitute a group of genes most appropriate for phenogenetic studies, since they seem to be relatively easily accessible to investigation with chemical methods. In the case of the gene *a*, the affected pigments have been characterized chemically by BECKER (1939, 1942) and distinguished under the name "ommochromes" from the melanin pigments contained in the exoskeleton. It has been shown by injection experiments that these pigments are dependent for their development on the presence of kynurenin (BUTENANDT, WEIDEL, and BECKER 1940a) and that this substance is apparently missing in *aa* individuals. Kynurenin seems to act as a precursor for the ommochrome pigments (BECKER 1939; KIKKAWA 1941; KÜHN and BECKER 1942).

The action of the gene *a* on pigment characters is therefore due to the fact that the precursor kynurenin is not formed. From chemical considerations as well as from comparison with mammals (KOTAKE and collaborators) it is generally accepted that kynurenin is ordinarily formed by oxidation of tryptophane in a series of reactions:



tryptophane α-oxy-tryptophane



kynurenin

Since kynurenin is not formed if *a* is present in homozygous condition, it has been concluded that this series of reactions is inhibited by the gene *a*.

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It has been claimed that the oxidation of tryptophane to α-oxy-tryptophane is the actual step which becomes impossible in *aa*, since α-oxy-tryptophane has been shown to cause pigment formation in *aa* Ephestia after injection (BUTENANDT, WEIDEL, and BECKER 1940b).

There are, however, other possibilities to account for the lack of kynurenin in *aa* Ephestia. Besides the oxidation to kynurenin, two more ways of oxidative breakdown of tryptophane have been described as occurring, both of them involving deamination. One of them leads through indolepropionic acid and indolelactic acid to indole, the other one through indolepyruvic acid and amino-benzoyl-pyruvic acid to kynurenic acid. If these mechanisms should occur in insects, they would be in competition with each other, and a shift toward greater velocity in one of the others should lead to a deficiency in the amount of kynurenin formed, and consequently to a reduction in the amount of ommochrome pigments present.

Furthermore, kynurenin, when formed in mammals, is further oxidized to other compounds, especially anthranilic acid and kynurenic acid. Whether or not reactions of this type actually occur in insects has not been definitely established, but such reactions have been used by BEADLE and TATUM (1941) to account for the effect of the gene "suppressor of vermilion" in Drosophila. If a reaction of this type should be speeded up in an insect, lack of kynurenin with consequent lack of eye pigments would result. This would constitute a third explanation for the action of the gene *a* in Ephestia.

In order to distinguish between these possibilities, tryptophane determinations in a^+a^+ and *aa* Ephestia were undertaken. If lack of kynurenin was due to oxidation of tryptophane to a compound other than kynurenin, or to further oxidation of kynurenin, no increase in tryptophane content would be expected in *aa* animals. If, on the other hand, the oxidation of tryptophane to kynurenin was inhibited, the unoxidized tryptophane or its derivatives should be found either in the tissues or in the excretions. Preliminary experiments (CASPARI 1943) have demonstrated that a significant increase of a substance giving the Hopkins-Cole reaction and the tryptophane reaction with p-dimethylaminobenzaldehyde is found in the tissues of *aa* animals, as compared with a^+a^+ Ephestia. In the present paper, evidence is given that this substance is actually tryptophane and that this increase in tryptophane content is accompanied by important differences in the chemical makeup of wild type and mutant animals.

The author wishes to acknowledge valuable aid and advice received from DR. DAVID R. GODDARD of the UNIVERSITY OF ROCHESTER, and from DR. ANDREW VANHOOK, formerly of LAFAYETTE COLLEGE.

MATERIALS AND METHODS

For the determinations, highly inbred strains of a^+a^+ and *aa* Ephestia, reared in fingerbowls on yellow corn meal, were used. Isogenic strains were not available. The animals were crushed in a mortar, dried for three days in a desiccator, over H_2SO_4 or $CaCl_2$ in vacuo, and then pulverized in a mortar. They were then kept in a desiccator over H_2SO_4 or $CaCl_2$ in vacuo until use,

but at least for one month. Males and females were usually, but not in all experiments, treated separately.

The amount of water lost by Ephestia imagoes by this treatment will be seen in table 1. Imagoes from a number of *aa* cultures were isolated on the day of hatching and either crushed and dried immediately or kept up to ten days in empty fingerbowls, and crushed and dried after this period. The weight of each batch before and after drying was taken. From the table it appears that the males are lighter than the females. Both sexes lose in weight with increasing age, due to the fact that they are unable to feed. In the males, the water content is constant up to eight days after eclosion and is consistently lower than in females. In the females, the relative water content increases after the fourth day, possibly because of the loss of dry matter through egg laying, which reaches its peak on the third and fourth day. The increased

TABLE 1

Water content of aa imagoes at different times after eclosion.

SEX	AGE IN DAYS	WET WEIGHT PER MOTH IN MG	DRY WEIGHT PER MOTH IN MG	PERCENTAGE WATER
♀	1	22.0	5.8	73.8
	3–4	20.4	5.5	73.2
	5–6	17.5	3.8	78.3
	7–8	15.9	3.6	77.4
	9–10	14.1	4.6	67.4
♂	1	14.8	4.4	70.3
	3–4	15.4	4.7	69.5
	5–6	13.4	4.0	70.1
	7–8	12.5	3.8	69.6
	9–10	11.4	4.1	64.0

loss of water in the oldest animals in both sexes cannot be explained. The animals used for the experiments were one to four days old. It may be concluded that about 5.5 mg dry substance corresponds to one female, and 4.5 mg dry substance to one male.

In one experiment, a^+a^+ and *aa* larvae were used. In this case, the sexes could not be distinguished in the *aa* strain, and therefore both sexes were dried and treated together. It proved to be impossible to dry the larval material to weight constancy by keeping it for several months over conc. H_2SO_4 in vacuo, and by heating it over night to 100°C. It was therefore used after keeping the a^+a^+ and the *aa* samples for over one year in the same desiccator over conc. H_2SO_4 in vacuo, assuming that after such a time the degree of desiccation would be more or less comparable.

In experiments involving + and *v* Drosophila, isogenic *bw* and *v bw* strains obtained through the courtesy of DR. G. W. BEADLE were used. The sexes were not separated. The animals were dried and pulverized according to the same procedure outlined above for Ephestia imagoes.

For the determination of tryptophane, three colorimetric methods were tried. The method of MAY and ROSE (1922), using p-dimethylaminobenzaldehyde, showed an increase of color developed in *aa* as compared with a^+a^+, which may indicate an increase in tryptophane or a related substance in *aa* (CASPARI 1943). The method was not convenient, however, for the present purpose, since relatively large amounts of material were necessary, and since a yellow color developing in a^+a^+ samples, possibly due to kynurenin (KIKKAWA 1941), interfered with the colorimetric determinations. Furthermore, tryptophane in this determination gives different reactions, depending on whether it is free or bound in proteins (SHAW and MCFARLANE 1940). A difference in this reaction may therefore be due to differences in the state of tryptophane rather than to different amounts.

The method of LUGG, as given by BLOCK and BOLLING (1940) involving alkaline hydrolysis, precipitation of tryptophane by mercuric sulfate, and a color reaction with phospho-molybdo-tungstic acid, gave higher amounts of tryptophane than the two other methods used, and no differences between a^+a^+ and *aa*. It may well be that with this method both tryptophane and kynurenin were determined, since kynurenin is precipitated by mercuric sulfate (KIKKAWA 1941) and the color reagent is rather unspecific.

All determinations reported in this paper were obtained by use of the Hopkins-Cole reaction with glyoxylic acid and H_2SO_4 as prescribed by BLOCK and BOLLING. The readings were done in a photoelectric colorimeter against a blank prepared in the same way as the determination without addition of glyoxylic acid, and the amounts determined with a calibration curve. The colorimeter used at LAFAYETTE COLLEGE was a Zenco Photelometer with green filter (maximum transmission 530 mμ). At the UNIVERSITY OF ROCHESTER, a Klett-Summerson photoelectric Colorimeter with Corning filters 9,780 S and 3,480 X, was used. In all cases, double or triple determinations were made of the same sample. The amounts of material used contained 0.01–0.1 mg tryptophane in 0.5–2 cc solution. Tryptophane values outside of this range were discarded, since the method in this case was not reliable.

THE TOTAL TRYPTOPHANE CONTENT OF EPHESTIA IMAGOES

Sixty mg pulverized dried material from Ephestia imagoes, males and females separately, was dissolved over night in 5 percent NaOH in a water bath at 56°C. In the morning, the dark brown fluid was centrifuged off the insoluble residue, and aliquots of this solution were taken for tryptophane determination with the Hopkins-Cole reaction. The substance determined by this procedure will be called "tryptophane" in this paper, although other substances carrying an indole ring may be involved. Data confirming the identity of this substance with tryptophane will be given in a later section of this paper. The results are given in table 2.

It is found that in both strains the males contain consistently more tryptophane than the females. This difference is significant in a^+a^+ ($t = 4.08$, $N = 20$, $P < .01$), insignificant in *aa* ($t = 0.58$, $N = 14$, $P > .05$).

In both sexes, *aa* material contained more tryptophane than a^+a^+ material.

This difference is highly significant in the females ($t=3.59$, $N=17$, $P<.01$), only barely significant in the males ($t=2.58$, $N=17$, $P<.05$).

It may be argued that this difference in tryptophane content between the two strains may be due to hereditary factors other than a^+/a. This objection cannot be refuted with certainty, since isogenic strains of a^+a^+ and *aa* Ephestia do not exist. In order to obtain some pertinent information, F_2 animals from crosses $a^+a^+ \times aa$ were tested. The results from these determinations are given in table 2.

It appears that both in F_1 and in the two F_2 types, the differences between the two sexes observed in the original strains have disappeared. In *aa* from

TABLE 2

Percentage tryptophane content of different genetic constitutions of Ephestia imagoes.

GENOTYPE	SEX	% TRYPTOPHANE ± SE
a^+a^+	♀	0.206 ± .019
aa	♀	0.335 ± .033
a^+a^+	♂	0.297 ± .008
aa	♂	0.360 ± .024
a^+a	♀	0.210 ± .012
a^+a	♂	0.191 ± .008
a^+- (F_2)	♀	0.205 ± .039
aa (F_2)	♀	0.359 ± .048
a^+- (F_2)	♂	0.224 ± .018
aa (F_2)	♂	0.304 ± .025

F_2, the females contain even more tryptophane than the males, though this difference is not significant ($t=1.44$, $N=9$, $P>.05$). This is due to a consistent lowering of the relative amount of tryptophane in the males, whereas the values obtained from the females are apparently not different from the original strains. The difference in tryptophane content between the original a^+a^+ males and the F_1 males is highly significant ($t=9.63$, $N=14$, $P<.01$), as is the difference between the original a^+a^+ males and the phenotypically wild type F_2 ($t=4.09$, $N=15$, $P<.01$). The difference between the original *aa* males and the *aa* males from F_2 is of the same order of magnitude as in the wild type males, but not significant ($t=1.55$, $N=12$, $P>.05$). It is not certain how this reduction in the relative amount of tryptophane in F_1 and F_2 males, as compared to the original strains, is to be explained. The hybrid combination of certain genetic factors may be involved. It is also possible that environmental factors have played a rôle, since the material from the original strains and from the crosses was collected at different times. Throughout this paper, it should be borne in mind that samples collected at different times and under different conditions cannot be easily compared. Any change in composition,

not only in proteins, but also in other constituents, may affect the tryptophane values obtained. For instance, better nourishment and consequent storage of fat would lower the relative amount of tryptophane which throughout this paper is expressed as percentage of dry weight.

In the heterozygous F_1 females, the tryptophane content is equal to that of the original a^+a^+ strain, whereas in the males it is even lower. No shift in the direction which would be expected if *a* were active in heterozygous condition is therefore present, and the phenotypically wild type animals which consist of a^+a^+ and a^+a may legitimately be considered together. With respect to tryptophane content, a^+ is completely dominant over *a*.

In F_2, both the wild type males and females give tryptophane values identical with F_1. The *aa* animals in both sexes contain an increased amount of tryptophane as compared to their wild type sibs. This difference is slightly significant both in the males ($t=2.58$, $N=10$, $P<.05$) and in the females ($t=2.51$, $N=9$, $P<.05$). It may therefore be considered as real.

Since the difference in tryptophane content between a^+- and *aa* Ephestia is maintained in F_2, it must be due to the gene *a* itself, or to a gene linked to it. In view of the fact that the haploid chromosome number of Ephestia is 30 (WAGNER 1931), the probability of two factors affecting tryptophane metabolism being situated in the same chromosome seems to be remote. Considering that an increase in tryptophane content in *aa* animals would constitute a logical consequence of the supposed action of the gene *a*, it is not unreasonable to conclude that the increase in tryptophane content in *aa* animals is due to the gene *a* itself.

THE EFFECT OF THE GENE *v* ON TRYPTOPHANE CONTENT IN DROSOPHILA

In Drosophila, the gene *v* acts similarly to *a* in Ephestia, since it also causes lack of kynurenin leading to lack of pigment in the eye. Because of this similarity in the action of *a* and *v*, it has been suggested (BEADLE, ANDERSON and MAXWELL 1938; BECKER 1938; KIKKAWA 1941) that these two genes are homologous. In order to obtain more information concerning this theory, the tryptophane contents of *bw* and *v bw* Drosophila were compared.

The results are given in table 3. Since in Drosophila separation of the males and females was not attempted, they are compared to Ephestia samples in which the sexes had not been separated either. It appears that there is no difference between *bw* and *v bw* Drosophila, whereas the difference between a^+ and *a* Ephestia is highly significant ($t=5.92$, $N=26$, $P<.01$).

This may be interpreted to mean that the mechanisms by which lack of kynurenin is caused in *a* Ephestia and in *v* Drosophila are different. In *a* Ephestia, the storage of non-converted tryptophane in the organism suggests inability to convert tryptophane into kynurenin, while in *v* Drosophila the other possibilities suggested earlier in this paper remain open. It is possible, however, that other differences in the metabolism of Ephestia and Drosophila are the cause for this difference.

Among these, the fact has been considered that adult Drosophila feed, whereas Ephestia is unable to feed from the moment of pupation on, since in Ephestia imagoes the midgut is closed against the foregut and therefore non-functional (BLAUSTEIN 1935). It could be imagined that a slight difference between + and *v* Drosophila is overshadowed by the constant intake of tryptophane containing food and excretion of its breakdown products. For this reason, the tryptophane content of the feeding stage in Ephestia, the larva, has been compared for the two strains.

The tryptophane values given in table 3 for Ephestia larvae are much lower than those for imagoes. This is due to the fact that the larval material still contained a certain amount of water and that a larger amount of fat is probably present, since part of the energy necessary for metamorphosis is derived from the breakdown of fats. The difference in tryptophane content between a^+a^+ and *aa* larvae is in the same direction as in the adults and is highly significant ($t=4.09$, $N=17$, $P<.01$). The non-existence of a difference in tryptophane content between + and *v* Drosophila cannot be due, therefore, to the fact that it feeds.

TABLE 3

Percentage tryptophane content of Ephestia and Drosophila imagoes and of Ephestia larvae. Both sexes combined.

SPECIES	STAGE	GENOTYPE	% TRYPTOPHANE ± SE
Ephestia	imago	a^+a^+	0.229 ± .015
Ephestia	imago	*aa*	0.409 ± .026
Drosophila	imago	*++bw bw*	0.217 ± 018
Drosophila	imago	*vv bw bw*	0.217 ± .033
Ephestia	larva	a^+a^+	0.095 ± .011
Ephestia	larva	*aa*	0.145 ± .011

These observations, though not adding evidence to the supposed homology of *a* in Ephestia and *v* in Drosophila, should not be interpreted as disproving it. It is possible that the non-oxidized tryptophane is excreted in Drosophila but stored in Ephestia. The result, however, points to an inherent difficulty in the establishing of gene homologies by the method of reciprocal transplantations.

Transplantations between different strains of one species can establish only the presence or absence of diffusible substances which may be correlated with genic differences between the strains. Similarly, cross transplantations between different species will indicate differences in the quantity of diffusible substances present. If different mutants in different species are tested, the result will indicate whether or not the same diffusible substance is affected. In this way, the results of transplantation experiments can frequently exclude homologies for phenotypically identical genes in different species by indicating either that the two genes act on different diffusible substances or that one of them acts on a diffusible substance and the other one does not. If, however, both genes act on the same diffusible substance, the differences induced may or

may not be effected by the same biochemical changes. In the latter case, homology of the two genes would appear extremely doubtful.

Kynurenin-deficient mutants have been described for different species of Drosophila, for *Ephestia kühniella* and for *Bombyx mori*. In all of them, a reduction in the pigmentation of the eyes and of some other organs is found. This is due to the fact that all through the insects, and probably in all Arthropoda, ommochromes are present as eye pigments (BECKER 1942). Since all these substances require kynurenin as a precursor, it is obvious that any reduction of kynurenin below a certain level will lead to pigment reduction. If genes inducing reduction of the amount of kynurenin present are to be considered as homologous, it will be necessary to prove that the mechanism causing lack of kynurenin is identical for those genes. That actually different mechanisms of inhibition of kynurenin formation may occur is suggested by the fact that besides *v* some other eye color mutants in Drosophila show reduced production of kynurenin, especially *ca* (CLANCY 1942).

FREE TRYPTOPHANE AND PROTEIN TRYPTOPHANE IN a^+a^+ AND *aa* EPHESTIA

The Hopkins-Cole reaction is not specific for tryptophane. It is considered to be a reaction of the indole ring, although some compounds possessing this structure do not give a positive reaction. The results reported above do not prove that in *aa* animals tryptophane is stored as such, but show only the presence of a substance containing the indole ring. In order to decide whether the substance is actually tryptophane or not, attempts were made to determine the tryptophane present in protein and non-protein fractions.

Samples of from 600 to 900 mg of dried material were extracted over night with 10 percent NaCl solution in a water bath at 56°C. The residue was removed by centrifuging, and the proteins in the solute precipitated with trichloroacetic acid. The supernatant was centrifuged off (fraction 1) and the precipitate dissolved in 5 percent NaOH (fraction 2). Since fraction 2 was always rather small, it was concluded that most of the proteins remained in the residue from the NaCl extraction. This residue, therefore, after being thoroughly washed, was subjected to digestion with pepsin for 24 hours and with a pancreatin preparation for another 24 hours in an incubator at 37°C. The solutions obtained as a result of these digestions were filtered off the residue and united to form fraction 3. The residue was then treated with 5 percent NaOH at 56°C over night, giving a fraction 4. The final residue was discarded. All operations were performed under addition of toluene to inhibit bacterial decomposition.

Four fractions were therefore obtained. Fraction 1 represents the non-protein fraction, fraction 2 and 3 protein fractions. Fraction 4 is chemically not easily defined, containing possibly some proteins not attacked by the enzymes used, part of the chitin, since the endocuticle is soluble in 5 per cent NaOH (BREDENKAMP 1942), perhaps some melanin and other substances which are not easily soluble. From all fractions, samples were taken for the determination of tryptophane with the Hopkins-Cole method. Furthermore.

in aliquots of fractions 1, 2 and 3, the amount of nitrogen was determined with a semimicro-Kjeldahl method. This was omitted in fraction 4, since it was assumed that chitin was present. Since chitin and other possible constituents of fraction 4 contain nitrogen, the nitrogen content of this fraction would be no indication of its protein content. In the determinations, samples of 1 to 5 cc, containing from 0.25–2.5 mg nitrogen, were used. In fraction 3, the values obtained were corrected for the tryptophane and nitrogen content of pepsin and pancreatin, as determined in blanks.

On the whole, three samples each of a^+a^+ females, *aa* females, and a^+a^+ males and two samples of *aa* males were fractionated and tryptophane and nitrogen determined. Each measurement was done in duplicate or triplicate, except in the case of fraction 2, where the material available was barely sufficient for single determinations. The results of these determinations are given in table 4. The total amounts of tryptophane given in table 4 are not significantly different from those given in table 2 for the females (0.229 percent for a^+a^+ and 0.318 percent for *aa*). For the males, it is lowered beyond the range found originally in the pure strains (table 2, lines 3 and 4) and it is for *aa* males with 0.289 percent inside the range found for *aa* males in F_2 (table 2, line 10: 0.304±.025). For the a^+a^+ males (0.143 percent±.006), it is decreased significantly below the level of F_1 males and of wild type males in F_2 ($t=4.95$, $N=7$, $P<.01$ and $t=4.28$, $N=9$, $P<.01$). Since here a similar reduction of tryptophane content of the males of the pure strains is found, as has been reported previously for the hybrids (see table 2), it must be concluded that environmental factors caused the increase in tryptophane content in the males over the females recorded in table 2, lines 1–4.

TABLE 4

Percentage tryptophane and nitrogen of different fractions of a^+a^+ and aa Ephestia.

	FRACTION	a^+a^+ ♀	*aa* ♀	a^+a^+ ♂	*aa* ♂
Tryptophane	nonprotein	0.059±.004	0.072±.011	0.018±.002	0.040±.006
	protein 1	0.009±.002	0.016±.002	0.007±0	0.016±.003
	protein 2	0.126±.018	0.178±.011	0.085±.004	0.209±.022
	protein (?) 3	0.035±.013	0.052±.005	0.033±.004	0.024±.005
Nitrogen	nonprotein	2.80±.15	2.42±.14	1.58±.07	1.53±.07
	protein 1	0.22±.05	0.34±.02	0.22	0.27
	protein 2	2.13±.08	2.61±.14	3.87±.16	3.56±.10
Tryptophane / Nitrogen	nonprotein	0.021	0.030	0.011	0.026
	protein 1 and 2	0.057	0.064	0.029	0.061

Values for a compound giving a positive reading as compared with the blank are found in every fraction examined. The color developed in fractions 2 and

3 is certainly due to tryptophane, since both fractions are protein, and tryptophane is the only compound giving a positive Hopkins-Cole reaction known to occur in proteins. The same probably holds true for fraction 4 in which some proteins which are not attacked by pepsin and pancreatin may be contained. Doubt attaches, however, to the findings in the nonprotein fraction. By observation with the naked eye, no color difference between determinations and blanks was observed in this fraction, but frequently there appeared a faint but definite cloudiness in the determinations, but not in the blanks. In some cases, the cloudiness was missing, and in these there was frequently, but not always, no difference in reading between the determinations and the blank. The values for nonprotein tryptophane are therefore certainly too high, and it is doubtful whether or not a measurable amount of nonprotein tryptophane was present in the samples. No cloudiness was observed in any of the other fractions.

In comparing the tryptophane content of a^+a^+ and *aa* material, it is seen that in all fractions and in both sexes *aa* material contains more tryptophane than a^+a^+ material. The only exception is fraction 4 (protein 3) in the males. Here the amount found in *aa* is smaller than in a^+a^+, but not significantly so ($t=1.41$, $N=8$, $P>.05$). The difference in fraction 4 in the females is in the expected direction, but also insignificant ($t=1.23$, $N=10$, $P>.05$). In the nonprotein fraction 1, the difference between a^+a^+ and *aa* material is not significant in the females ($t=1.10$, $N=14$, $P>.05$), but significant in the males ($t=3.43$, $N=9$, $P<.01$). However, as indicated above, it seems doubtful if this difference can actually be ascribed to tryptophane.

At all events, the main difference in tryptophane content between the two strains is situated in the protein fractions 2 and 3. In fraction 2 this difference is slightly significant in both sexes ($t=3.46$, $N=7$, $P<.02$ for the males, $t=2.25$, $N=11$, $P<.05$ for the females). In fraction 3, which contains the highest absolute amounts of tryptophane, the difference between a^+a^+ and *aa* is highly significant in the males ($t=5.54$, $N=10$, $P<.01$), slightly significant in the females ($t=2.81$, $N=12$, $P<.05$). It must therefore be concluded that most if not all of the tryptophane by which *aa* material exceeds a^+a^+ is present in the proteins.

Three possibilities arise for this increase in tryptophane in the proteins. It may be due to a proportional increase in the amount of proteins present, or it may be that certain proteins rich in tryptophane are increased, possibly at the expense of others, or the tryptophane may be built into a completely new type of protein. In order to obtain information relating to this question, protein nitrogen was determined in fractions 2 and 3. Table 4 indicates in both strains considerable differences in nitrogen content between the sexes, both in nonprotein-nitrogen and in the main protein fraction 3. Between the two strains, no difference is found in the nonprotein-nitrogen. In the first protein fraction 2, the difference is just on the borderline of significance in the females ($t=2.45$, $N=6$, $P\cong.05$), while no attempt at evaluation has been made in the males, since only three measurements are available. In the main

protein fraction 3, the *aa* females show a slightly significant increase in nitrogen content ($t=2.95$, $N=12$, $P<.05$), whereas the *aa* males show an insignificant decrease as compared with the corresponding a^+a^+ material ($T=1.64$, $N=8$, $P>.05$).

The combined tryptophane content of the two protein fractions expressed as the fraction tryptophane/nitrogen is given in the last line of table 4. It turns out that in both sexes the relative tryptophane content of the proteins appears to be increased in *aa* animals as compared to a^+a^+ animals. The increase is very small in the females, considerable in the males. The possibility that the increased amount in protein tryptophane may be caused by a concomitant increase in the amount of protein present seems therefore unlikely. It must be concluded that either a different kind of protein is formed in *aa* animals or that a protein rich in tryptophane may be increased in amount at the expense of other ones poor in tryptophane.

Because of the importance of this conclusion, the experiment was repeated in a somewhat different fashion. Samples of 300 mg dried and pulverized material were extracted with boiling ether in a Soxleth for four hours. They were dried at 70°C and the residue weighed. The residue was then treated with n/10 NaOH in the cold for one hour, in order to dissolve the proteins without hydrolizing them. The samples were ground for ten minutes in n/10 NaOH in a homogenizer after POTTER and ELVEHJEM which was driven by a motor, then kept for 40 minutes in ice water, finally ground another ten minutes in the homogenizer. At the end of this procedure, the solution was strongly alkaline (p_H above 10) as tested with hydrion paper. The fluid was centrifuged off the residue, and the proteins precipitated with cold trichloroacetic acid. The precipitate was separated by centrifuging and dissolved in 5 percent NaOH. The residue from the n/10 NaOH extraction was again extracted with 5 percent NaOH at room temperature over night. The supernatant was centrifuged off and the residue washed with distilled water to remove the NaOH until neutral reaction, dried and weighed.

In this way, not counting the ether extract and the final residue, three fractions were obtained, the nonprotein fraction, a protein fraction, and a third fraction containing some rather insoluble proteins besides other substances. In these three fractions, tryptophane was determined according to the Hopkins-Cole method. In the nonprotein fraction and in the first protein fraction, nitrogen was determined with a micro-Kjeldahl method. Samples of 2 to 4 cc of the nonprotein fraction and of 1 cc of the protein fraction were used in this experiment for the nitrogen determinations. These samples contained 0.13–0.42 mg and 0.49–1.00 mg nitrogen, respectively.

Six samples each of a^+a^+ males and of *aa* females, and five samples each of a^+a^+ females and *aa* males were examined. In six cases, paired observations were made. Samples of a^+a^+ and *aa* material of the same sex were extracted and fractionated at the same time side by side, and the measurements of the fractions were done simultaneously.

A difference between a^+a^+ and *aa* proteins could easily be seen immedi-

ately after precipitation: the protein of the *aa* material had a whitish-gray color, whereas the protein from a^+a^+ animals was distinctly pink. This difference suggests the presence of chromoproteins in a^+a^+ but not in *aa* material. This may tend to confirm the hypothesis put forth by BECKER (1942) that the ommochromes are present in the animal in combination with proteins. In a^+a heterozygotes the pink color of a^+ protein was completely dominant.

The results of the tryptophane and nitrogen measurements of the different fractions are given in table 5. If the values are compared to those reported in table 4, a number of striking differences are revealed. The total amounts of tryptophane are increased in every sample above those found in

TABLE 5

Percentage tryptophane and nitrogen content of different fractions of a^+a^+ and aa Ephestia.

	FRACTION	a^+a^+ ♀	*aa* ♀	a^+a^+ ♂	*aa* ♂
Tryptophane	nonprotein	0.027 ± .007	0.019 ± .004	0.026 ± .005	0.040 ± .007
	protein 1	0.240 ± .018	0.280 ± .017	0.219 ± .017	0.249 ± .021
	protein 2	0.076 ± .006	0.094 ± .007	0.110 ± .008	0.179 ± .009
Nitrogen	nonprotein	0.540 ± .030	0.523 ± .023	0.485 ± .022	0.341 ± .032
	protein 1	1.315 ± .042	1.280 ± .063	1.120 ± .025	1.105 ± .089
Tryptophane / Nitrogen	nonprotein	0.050	0.036	0.055	0.117
	protein 1	0.183	0.215	0.192	0.213

the earlier samples. No explanation for this finding can be given. However, it may be suggested that differences in culture conditions may be responsible. The experiments reported previously were done in Easton, Pa., where the animals were reared in an incubator at 18°C. The last experiment was done in Rochester, N. Y., and the animals were kept at room temperature in summer, usually close to 25°C. The animals develop faster at higher temperature and do not reach the same size as at 18°C. If a larger part of this difference in size was due to substances other than protein, a larger relative content of tryptophane at the higher temperature would be understandable. It is, possible, however, that other differences in climate or culture conditions may have been responsible.

The amount of nonprotein nitrogen is decreased about 70–80 percent as compared to that reported in table 4. This indicates that a large amount of nonprotein nitrogen has been removed by the ether extraction. The nonprotein nitrogen remains higher in the females than in the males.

The amount of protein extracted with n/10 NaOH is smaller than that found previously, as indicated by the amount of nitrogen, but the tryptophane found in the protein fraction is considerably increased. The relative amounts of tryptophane, as expressed by the fraction tryptophane/nitrogen are therefore several hundred percent larger than those found in the earlier experiment.

A difference in the total tryptophane content between the two sexes is found in this instance, just as in the first experiment (table 2). It is slight in the a^+a^+ material (0.343 percent for the females, 0.353 percent for the males), much higher in the *aa* animals (0.378 percent for the females, 0.455 percent for the males).

In spite of all these differences from the results obtained earlier, the *aa* samples contain in every fraction more tryptophane than the corresponding a^+a^+ samples. The only exception is the nonprotein fraction in the females, but the lack of validity of the data for nonprotein tryptophane has been pointed out before. In all protein fractions, the *aa* material is richer in tryptophane than the corresponding a^+a^+ material, but this difference is significant for the second protein fraction of the males only ($t=5.89$, $N=19$, $P<.01$).

Although the differences between the amounts of tryptophane found in the protein fractions 1 are not statistically significant ($t=1.09$, $N=22$, $P>.05$ for the males, $t=1.62$, $N=23$, $P>.05$ for the females), they are probably real. This is indicated by the fact that in both sexes the amount of protein nitrogen is somewhat lower in *aa* than in a^+a^+. The relative amount of tryptophane in the proteins, as expressed by the fraction tryptophane/nitrogen is therefore increased in both sexes in *aa* over the values found for a^+a^+. No differences between the sexes are apparent.

TABLE 6

Ratio tryptophane/nitrogen in the protein fraction 1 in six pairs of measurements.

SEX	a^+a^+	*aa*	DIFFERENCE
♂	0.230	0.236	0.006
♂	0.194	0.230	0.036
♀	0.201	0.235	0.034
♀	0.174	0.202	0.028
♀	0.218	0.251	0.033
♀	0.231	0.252	0.021
mean	0.208	0.234	0.026

As mentioned above, six pairs of parallel observations are available for this set of experiments. They involve both males and females. In each of these pairs of data, the *aa* sample contains a larger amount of tryptophane, relative to the protein nitrogen, than the corresponding a^+a^+ sample. The data for these six pairs of measurements are given in table 6. If the mean of the differences is tested for its deviation from 0, a t value of 5.709 for 5 degrees of freedom is obtained. This value of t is below the 1 percent level. It seems highly probable therefore that in this experiment, as well as in the preceding one, the amount of tryptophane in the protein fraction was higher in *aa* material than in a^+a^+ material.

THE AMOUNTS OF ETHER EXTRACTABLE MATERIAL AND RESIDUE IN a^+a^+ AND *aa* MATERIAL

The experiment summarized in table 5 and 6 gave two more sets of measurements which may be compared, besides the different tryptophane and nitrogen fractions. The first is the difference in weight between the sample before and after ether extraction, comprising the ether soluble fraction. The other one is the residue remaining after extraction with 5 percent NaOH. It consists probably mainly of chitinous material, since the exocuticle is insoluble in alkalies (BREDENKAMP 1942). The values obtained for these two fractions are given in table 7.

TABLE 7

Percentage total ether extract of a^+a^+, a^+-, and aa material per milligram dry weight, ± standard error.

GENOTYPE	SEX	
ether extract		percentage ether extract ± SE
a^+a^+	♀	36.2±0.6
aa	♀	30.9±1.0
a^+a^+	♂	34.9±1.3
aa	♂	31.9±1.0
a^+- (F_2)	♀	31.3±0.7
aa (F_2)	♀	29.9±2.4
a^+- (F_2)	♂	31.9±2.6
aa (F_2)	♂	28.9±3.4
final residue		percentage residue ± SE
a^+a^+	♀	10.4
aa	♀	11.8
a^+a^+	♂	9.5±0.6
aa	♂	12.5±1.0
a^+- (F_2)	♀	13.7±0.7
aa (F_2)	♀	12.8±0.6
a^+- (F_2)	♂	11.9
aa (F_2)	♂	12.1

In the ether-soluble fraction, there is no difference between the sexes, but a striking difference between the two strains, a^+a^+ material containing more ether-soluble material than *aa* material. The difference is significant in the females ($t=5.25$, $N=11$, $P<.01$), not significant in the males ($t=1.84$, $N=11$, $P>.05$). Six paired comparisons of a^+a^+ and *aa* material extracted at the same time gave highly significant differences ($t=16.42$, $N=5$, $P<.01$). It seems certain, therefore, that the wild type samples contained more ether extractable material than the mutant samples.

In the residue, there is again no difference between the sexes, but in both sexes a difference between the two strains, *aa* giving more residue than a^+a^+. This difference is not significant in the females, where only two measurements for each strain are available. For the males, where four measurements for a^+a^+ and five for *aa* have been done, the difference is significant at the 5 percent

level ($t=2.53$, $N=8$, $P<.05$). Again, five sets of paired comparisons gave a highly significant difference, since in each pair *aa* gave higher values than the corresponding a^+a^+ material ($t=5.73$, $N=4$, $P<.01$). In the sixth set of paired determinations, one of the residue samples was lost.

These results have a certain bearing on the tryptophane determinations described above. Since all tryptophane determinations have been expressed as percentage dry weight, they will be affected by the different amounts of fats and residue present in the two strains. If the tryptophane content of a^+a^+ and *aa* material reported in table 5 is expressed in percentage extracted dry weight, instead of total dry weight, the a^+a^+ tryptophane will be more strongly increased relatively than the *aa* tryptophane, and therefore the difference in tryptophane content between a^+a^+ and *aa* will become smaller. The differences, however, are maintained in every comparison. Furthermore, this loss of significance will to a certain degree be counterbalanced by the increased amount of residue in *aa*.

These considerations do not affect the relative amounts of tryptophane in the proteins, as expressed in the last lines of tables 4 and 5 and in table 6, since both tryptophane and nitrogen were calculated as percentage total dry weight. The general conclusion that *aa* proteins contain more tryptophane than a^+a^+ proteins will therefore not be affected by the finding of differences in other fractions.

In order to determine whether these differences in ether-soluble material and in final residue were dependent on the gene *a*, a^+- and *aa* material from F_2 were tested. The a^+- and *aa* animals hatching from a number of F_2 cultures were ground up and dried separately. In all cases, paired determinations were done, an a^+- and an *aa* sample of the same sex being extracted at the same time side by side first for four hours with ether in a Soxleth apparatus, then over night at room temperature in 5 percent NaOH. The results are included in table 7.

It appears that no difference exists in F_2 between the amounts of residue obtained from a^+- and from *aa* samples. The insignificant differences found are in opposite direction in the two sexes.

The amounts of ether-extracted material are slightly less than those observed in the pure strains. The differences between a^+- and *aa* are less pronounced than in the pure strains, but in both sexes in the same direction. In six out of the seven paired samples, a^+- contained more ether-extractable material than *aa*, while in the seventh the difference was 0.1 percent dry weight in the opposite direction—that is, practically identical. The mean difference between the two genotypes was $2.13\pm.58$ percent, significant, at the 2 percent level ($t=3.65$, $N=6$, $P<.02$). It must be concluded that in F_2 the difference between the two genotypes is due to the gene *a* or a gene closely linked to it.

EVALUATION OF RESULTS

In evaluating the results, it must be considered that statistical significance is not necessarily an indication of reproducibility of a result. This can well be

seen in the difference in tryptophane content between males and females. It has been claimed previously (CASPARI 1943) that Ephestia males have a higher tryptophane content than the females, and the disagreement with the finding of DEMYANOVSKII (1938) that in *Bombyx mori* the females contain more tryptophane than the males has been pointed out. The basis for this claim was a significant (at the 1 percent level) difference between the sexes in a^+a^+ material, and an insignificant difference in the same direction in *aa* (see table 2, this paper). In three more independent experiments reported in this paper, only one showed this difference between the sexes. This difference therefore cannot be considered as being characteristic for *Ephestia kühniella*, but only as occurring in certain samples.

In all samples investigated, the tryptophane content of *aa* material was higher than in a^+a^+ material. This difference was statistically significant in some experiments, insignificant in others. Of 18 comparisons of groups of a^+a^+ and *aa* samples reported in tables 2–5, 16 showed a higher tryptophane content in the *aa* material than in the a^+a^+ material; in two comparisons the opposite was true. If both genotypes contained the same amount of tryptophane, such an outcome would have a probability of occurring by chance of .0002. It must therefore be concluded that an increase in tryptophane content is associated with the genotype *aa*.

The data furthermore indicate that most or all of the increased amount of tryptophane in *aa* material is contained in the protein fractions. This is borne out by the consistent differences in tryptophane content found in the protein fractions in the two sets of experiments reported in tables 4 and 5. It is also obvious from the fact that the total amounts of nonprotein tryptophane found are hardly sufficient to account for the size of the difference between a^+a^+ and *aa* material. Since no consistent differences in protein content between the two strains have been found, and since the relative amount of tryptophane has been consistently higher in *aa* than in a^+a^+ proteins, it becomes necessary to conclude that qualitative differences exist between the proteins of the two strains examined. It cannot be decided whether qualitatively different protein molecules are formed or whether certain tryptophane-rich proteins are increased in amount at the expense of other proteins poor in tryptophane. In either case, the general makeup of the cell proteins would be changed under the influence of the gene *a*.

With respect to the ether-extractable fraction which consists probably mainly of fat, two sets of measurements, the pure strains and F_2, gave higher amounts in a^+- than in *aa* material in both sexes. This appears to suggest a difference due to the gene *a*. This is especially true for F_2 where both the other genes and the environmental conditions were highly similar. It must be taken into account, however, that in F_2 the two types lived in food competition with each other, and that *aa* develops somewhat more slowly (KÜHN and HENKE 1930). The *aa* animals would therefore be at a disadvantage which might result in a reduced storage of fats. An attempt was made to avoid this difficulty by supplying optimal food conditions in F_2. Since competition was excluded in the samples from pure strains, the experiment seems

to suggest that the gene *a* might influence the amount of ether-extractable material stored also by means other than control of the speed of development.

DISCUSSION

In table 8, all effects known to be due to the gene *a* in homozygous condition are summarized. It is the task of physiological genetics to find the connections between these different characteristics as far as possible.

The morphological effects on pigmentation have all been shown to be a consequence of lack of kynurenin. The developmental activity of the gene *a* appears to be clear, as far as pigmentation is concerned. All a^+a^+ tissues investigated—testes (CASPARI 1933), ovaries (KÜHN, CASPARI, and PLAGGE 1935; DA CUNHA 1935), brain (KÜHN 1936), ventral nerve cord (RANZI 1939),

TABLE 8

Characters known to be affected by the gene a in homozygous condition. (Data from KÜHN *and* HENKE *1930, 1932;* CASPARI *1933;* PLAGGE *1935,* KÜHN, CASPARI *and* PLAGGE *1936,* CASPARI *1943.)*

CHARACTER	a^+a^+	aa
Pigmentation		
eyes	black	red
brain (optic ganglia and corpora pedunculata)	brown	light pink
testes	pigmented	colorless
larval ocelli	strongly pigmented	weakly pigmented
larval hypodermis	pigmented	colorless
proteins	pink	white
Chemical constitution		
kynurenin	present	absent or reduced
protein tryptophane		increased
ether extractable substances		reduced
General biological characters		
viability		reduced
speed of development		reduced

fat body (DE MELLO 1940), hypodermis (DE MELLO 1940)—have been shown to release a larger or smaller amount of kynurenin or of a related substance (KÜHN 1936). It seems probable that all a^+a^+ cells form this substance. Furthermore, it seems likely that kynurenin is formed at all stages of development. For the first kynurenin-controlled characters, the pigment of the larval ocelli and of the larval hypodermis are already formed in embryonic life, while release of kynurenin has been demonstrated by transplantation for both the larval and the adult testis (DA CUNHA 1935). A general survey of the development of kynurenin-dependent pigmentation in Ephestia would therefore indicate that kynurenin is formed by all cells of the body at any time and is therefore a normal product of the metabolism of the cell. Formation of pigment, as a result of the presence of kynurenin, is restricted to certain cells,

and these different types of cells have different definite sensitive periods in which they are able to react on kynurenin (PLAGGE 1936). This sensitive period of reactivity is due to the developmental state of the reacting tissue and has no relation to a possible "time of activity" of the gene a^+.

The gene *a* does not influence the reactivity of the tissues, as far as is known; its influence on pigmentation consists in the suppression or reduction of kynurenin formation in all cells at all times. The increase in tryptophane content in *aa* cells may then be taken to indicate that the enzymatic oxidation of the tryptophane to kynurenin is inhibited by the gene *a*, and that the unoxidized tryptophane is stored in the animal. The fact that the non-metabolized tryptophane is incorporated in the proteins is unexpected, since in similar cases in which a substance cannot be broken down in the normal way it is found to be excreted in the urine—for example, in alcaptonuria and in phenylketonuric idiocy (BLOCK, JERVIS, and collaborators 1940). It may be pointed out, however, that in insects, where actual excretion is impossible during metamorphosis, the formation of certain pigments such as pterins and ommochromes may be regarded as the normal way of excretion of certain metabolic products—that is, their removal from active metabolism.

There is, however, still another possibility to account for the lack of kynurenin and the concomitant change in protein composition in the cells. It may be assumed that the primary action of the gene *a* affects the constitution of the proteins and that because of the different structure of the proteins in *aa* animals less tryptophane is available for transformation into kynurenin. This type of mechanism would consider differences in protein structure as the primary action of the gene *a*, and the non-oxidation of tryptophane as a secondary consequence. This would be in agreement with the theories of CASPERSSON who regards the synthesis of proteins as the main function of nucleic acids in the cell. No decision between the two possibilities suggested can be made on the basis of the material presented.

While it is evident that the absence of kynurenin and the increase of tryptophane in *aa* material must be related to each other, the relation of the decrease in amount of ether-extractable material to the other chemical characters remains obscure. It may be assumed that a situation of primary pleiotropism is present, but it seems just as likely that they are all in some way related to each other. The impression prevails that as a result of a single gene substitution $a^+ \rightarrow a$ the whole metabolic equilibrium of the cell is upset. The new condition expresses itself in the amount of ommochrome pigments, kynurenin, and ether-extractable materials formed, and in the amino acid composition of the proteins.

This conclusion may appear to be in disagreement with the conclusions reached by BEADLE and his collaborators (HOROWITZ, et al. 1945) in their studies on Neurospora. They found that every gene investigated controls one particular metabolic step only. It will appear, however, that the different, conclusions drawn are actually only different aspects of the same phenomenon due to different experimental techniques. BEADLE'S technique enables him to analyze the primary metabolic step which is inhibited by a gene mutation.

The secondary effects cannot be observed, since the mutations are lethal unless the missing metabolic product is supplied with the medium. In the experiments with Ephestia, the starting point is a simple recessive mutation, and the procedure adopted is to describe as fully as possible all changes due to the gene mutation and to investigate their possible connections with each other. It will be easily imagined that if a gene mutation suppresses a certain metabolic step, profound changes in other parts of metabolism are bound to occur. The unmetabolized compound, unless excreted, will stay in the cell and exert certain effects. The product of the metabolic step, on the other hand, will be missing, and all biochemical processes in which this substance may be involved will become impossible.

No evidence is available concerning the connection of the changes in viability and speed of development associated with the gene *a* to the biochemical changes. It would appear reasonable to consider them as correlated with one or several of the biochemical changes found in *aa* individuals.

SUMMARY

In *aa* Ephestia, both in imagoes and larvae, the tryptophane content is increased as compared to a^+a^+ Ephestia.

a^+a heterozygotes have the same tryptophane content as a^+a^+ animals. In F_2, *aa* material has the increased tryptophane content of the original *aa* strain, indicating that the increase in tryptophane content is caused by the gene *a*.

No differences in tryptophane content were found between *bw* and *v bw* Drosophila.

In *aa* material, most or all of the increased amount of tryptophane is contained in the proteins. The relative amount of tryptophane in *aa* proteins is increased. No difference in protein content between the two strains was found. a^+a^+ proteins are distinguished from *aa* proteins by a pink coloration.

In *aa* animals, the ether-extractable materials are reduced in amount as compared to a^+a^+ animals.

It is concluded that in *aa* animals, the oxidation of tryptophane to kynurenin is inhibited. This may be caused either by a direct inhibition of the oxidation process, the proteins being changed secondarily, due to an increased amount of tryptophane available, or primarily by a different structure of the proteins, causing tryptophane not to be available for kynurenin production.

It is concluded that as a result of the gene substitution $a^+ \rightarrow a$ the biochemical setup of the cell is profoundly changed.

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CYTOLOGICAL STUDIES IN THE MUSAE. II. MEIOSIS IN SOME DIPLOID CLONES

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INTRODUCTION

THE banana breeding program currently being carried out in Jamaica is largely based on the progeny of the triploid ($2n=33$) Gros Michel crossed with a number of naturally occurring diploids. The bulk of such progeny have been found to be tetraploid (LARTER 1935 and unpublished; CHEESMAN and DODDS 1942), which suggests that Gros Michel has a tendency to throw a majority of triploids among its viable gametes. The mechanism involved is presumably the formation of restitution nuclei or the suppression of one or the other of the two meiotic divisions. A cytological survey of relevant material has been carried out partly with the hope of finding evidence concerning this hypothesis and partly to lay a cytological foundation for more extensive cytogenetical and cytotaxonomical studies than have hitherto been carried out.

The first paper of this series (WILSON 1946) dealt with several triploids representing the three groups, Cavendish-Robusta, Gros Michel, and the plantains. The present paper deals with several species of diploids with special reference to the *Musa acuminata* group from which virtually all of the successful crosses have been made. The third paper will be largely concerned with the more extensive seedling families from the general cross Gros Michel × *M. acuminata*.

With reference to Gros Michel, the cytological findings were in agreement with the "restitution nuclei" hypothesis in so much as definite evidence of a tendency to suppression of the first division of meiosis was found. This of course presupposes normality on the part of the diploid male parents—an assumption borne out by the data to be presented below.

MATERIALS AND METHODS

All materials were obtained from the Botanist's collection of wild type Musae. Four species, *Musa acuminata* Colla, *M. rubra* (?), *M. Balbisiana* Colla, and *M. Nagensium* Prain, were used. Preparations were made by an acetocarmine squash technique following fixation in 3:1 alcohol-acetic acid. Permanent mounts were made by the venetian turpentine method (WILSON 1945). Observations and camera lucida drawings were made with Zeiss 10× and 20× oculars combined with Zeiss 1.3 mm, Baker 1/12″, and Watson 1/12″ objectives.

OBSERVATIONS

As in the previous paper, the observational data fall into the following groups: (1) pairing at first metaphase, (2) chromosome segregation, (3) the number of cells per "tetrad" and (4) pollen grain diameter.

TABLE 1

Meiosis in diploid Musae

VARIETY	FIRST METAPHASE		FIRST ANAPHASE		SECOND METAPHASE		SECOND ANAPHASE		NO. CELLS PER TETRAD						POLLEN GRAIN DIAMETER
	NORM.	OTHER	NORM.	OTHER	NORM.	OTHER	NORM.	OTHER	3	4	5	6	7	9	
Musa acuminata:															
ZA	28	0	7	1 (10–2–10)	10	1 (10/2+10)	9	0	—	100	—	—	—	—	58.7±.44
ZB	14	0	2	0	13	0	7	0	—	100	—	—	—	—	58.3±.19
ZC	25	0	—	—	19	0	4	1 (11–11/12–10)	—	99	1	—	—	—	57.5±.27
ZD	38	0	4	0	4	0	2	0	—	200	—	—	—	—	55.6±.19
*ZE	10	48	1	0	2	10	2	5	—	36	45	16	2	1	68.4±.71
ZG	16	0	8	0	—	—	2	0	—	100	—	—	—	—	63.2±.50
Calcutta 4	41	0	2	0	11	0	—	—	—	100	—	—	—	—	59.1±.15
Long Tavoy	39	2 (10II+2I)	16		35	1 (11/10+1)	2	1 (11–11/12–10) 2 (11–11/22)	—	101	3	2	—	—	56.0±.80
B6	24	0	4	0	16	0	5	0	—	99	1	—	—	—	59.0±.19
B7	41	1 (10II+2I)	6	0	7	0	11	0	—	100	—	—	—	—	61.4±.40
**Pisang Lilan	10	52	23	1 (10–12) 1 (10–11+1) 1 (11~11) 2 (10–2–10) 1 (10–1–11)	1	1 (10/1+11) 1 (10+1/11–11) 1 (12/10)	3	1 (10–10/12–12)	3	274	16	7	—	—	64.6±.65
M. rubra (?):															
B10	30	1 (10II+2I) 1 (9II+4I)	8	1 (10–10+1–1) 1 (11~11) 1(11–10+1)	37	1 (10+1/11) 1 (12/10)	8	1 (10–1–11/11–11) 1 (11–11/13–9)	—	98	—	2	—	—	61.6±.29
M. Balbisiana:															
Brachycarpa	43	1 (10II+2I)	10	1 (12–10) 1 (11–1–10)	26	1 (10/12)	6	1 (11–11/12–10)	—	100	—	—	—	—	54.9±.36
Philippine Unidentified	9	8 (10II+2I)	13	3 (10–12) 1 (11–3–8) 1 (11–1–10) 1 (11–2–9)	16	0	5	0	—	91	6	3	—	—	56.5±.25
Butuan	22	0	9	0	5	0	1	0	—	100	—	—	—	—	55.8±.27
TaNiPa	31	0	4	0	11	0	2	0	—	100	—	—	—	—	51.9±.22
M. Nagensium:															
B8	14	0	2	1 (scattered)	5	0	4	0	—	100	—	—	—	—	60.7±.27
B9	19	0	7	0	3	1 (11/10+1)	5	0	—	99	1	—	—	—	59.5±.21

* See table 2.
** See table 3.
~ = bridge.

Musa acuminata

All the varieties which proved most successful in crosses with Gros Michel came from this species. In the present study 11 varieties were used—namely, six of the Zebrina series, Long Tavoy and Calcutta 4 which are known to be related, B6 and B7 which appear to be identical, and Pisang Lilan, a female sterile diploid. Of these 11 varieties extensive use in crosses was made of ZA, ZB (Kewensis or K in the Breeding records), Calcutta 4, B6, B7 and Pisang Lilan.

Table 1 gives the essential figures for all stages of meiosis studied. With the exception of ZE (fig. E, U) and Pisang Lilan (fig. K), which show definite abnormalities, all the other varieties of this species may be considered normal diploids (fig. A–R) although several of them show occasional aberrations.

The figures given in table 2 indicate the type and extent of aberration in ZE.

TABLE 2

Zebrina "E"—univalent and bivalent frequency.

	NO. OF CELLS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Univalents																
Navel 1	18					1		3		5		1		7		1
" 2	18	7		10				1								
" 3	22	3		14		3		1		1						
Bivalents																
Navel 1	18					1	7	1	5	3	1					
" 2	18									1		10	7			
3	22							1		1	3	14	3			

Chromosome Segregation

NO. POLES	7	8	9	10	11	12	13	MEAN NUMBER LAGGING OR LOST
58	3	6	9	17	19	3	1	1.1

Three navels (male inflorescences) from a single clone were used, and all three show different degrees of abnormality. Since Navels 1 and 2 came from the same root, the difference is not likely to be genetical but must almost certainly be ascribed to slight environmental differences. In general there is a tendency to asynapsis or desynapsis involving usually a single pair in Navels 2 and 3 and from two to seven pairs in Navel 1. Faulty first anaphase separation conforms to expectation. That the two sets of chromosomes are sufficiently homologous to pair is shown especially by Navel 2 in which nearly half the first metaphase plates had 11 bivalents. Furthermore, in some cases, the structure of the univalents indicated that they had earlier been involved in pairing and were therefore desynaptic rather than asynaptic.

The figures for Pisang Lilan given in table 3 are most easily explained by the assumption that this variety is a reciprocal interchange hybrid (DODDS 1943). Excluding the two doubtful cases of quadrivalent formation, however, other explanations are possible, especially the assumption of duplication. To obtain such a high degree of trivalent formation, however, would require

a large duplication which would lead to definite unbalance, a condition which experience has shown does not generally function well in the banana.

Chromosome segregation is not seriously affected by this translocation (table 1) but a fair proportion (on random segregation of the trivalent and

TABLE 3

First metaphase associations in Pisang Lilan.

NO. CELLS	11II	10II+2I	1III+9II+1I	1IV+9II
62	10	18	32	2(?)

univalent), about two-thirds, of the apparently euploid gametes would be unbalanced and therefore probably inviable. Observation over a considerable period has shown that about half the pollen is infertile, which is in reasonable agreement with the cytological data. The complete female sterility of this variety, however, must be partially assigned to other causes presumably

FIGURES A–R.—First metaphase chromosomes of Zebrina "A," "B," "C," "D," "E," and "G"; Calcutta 4; Long Tavoy; B6; B7; Pisang Lilan; *Musa rubra* (?); Brachycarpa, Philippine unidentified; Butuan; TaNiPa; B8 and B9 respectively.

genetical, at least in the last analysis (see DODDS 1945 for discussion of sterile diploids).

Musa rubra (*?*)

Only one variety of this species was obtainable. This (B10) is a dwarf plant, shooting an erect stem surmounted by a bright red navel. With the exception of two cells showing asynapsis of a single chromosome pair and a few with slightly aberrant segregation, this variety is a perfectly normal diploid.

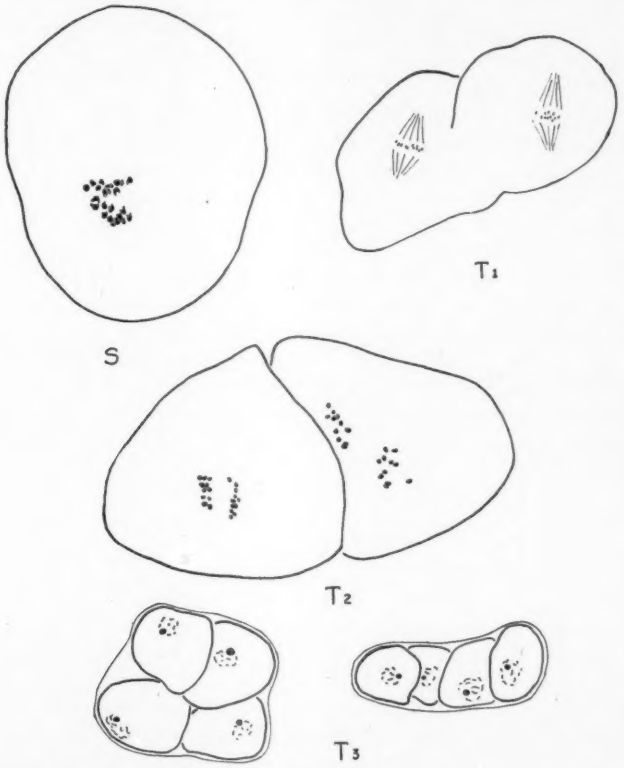

FIGURE S.—First anaphase of Zebrina "A."
FIGURE T1.—Second metaphase of Zebrina "C."
FIGURES T2, T3.—Second anaphase and tetrads of Zebrina "A."

Musa Balbisiana

Four varieties of this species were used. All of them are relatively tall plants with thick pseudostems, short fat fingers, and a tendency to retain the flower bracts on the stalk. Three of the four varieties (Brachycarpa, Butuan, and

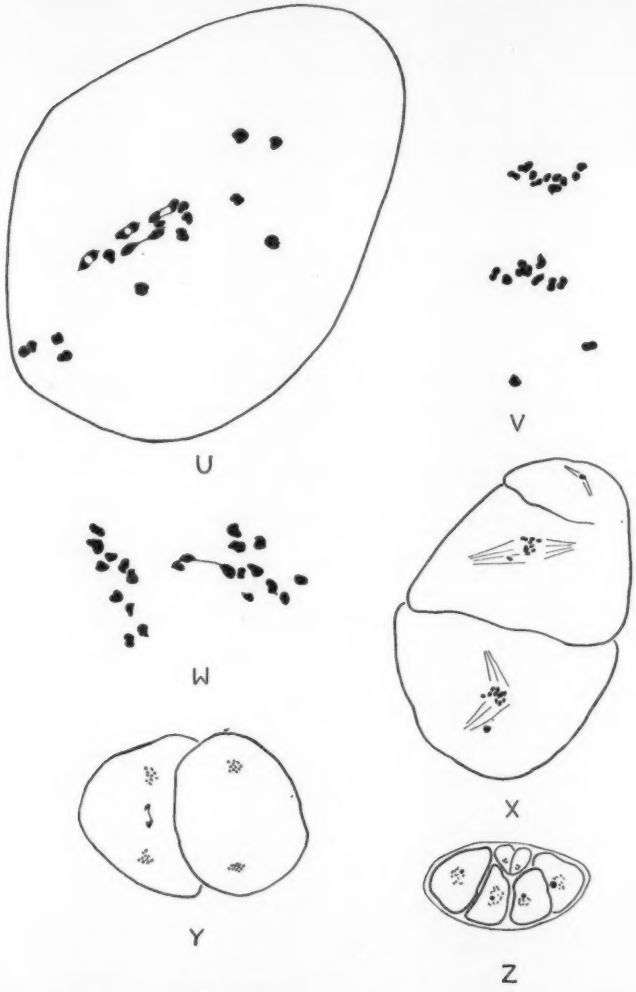

FIGURE U.—First metaphase of Zebrina "E."
FIGURE V.—Aberrant first anaphase of *Musa rubra* (?).
FIGURE W.—First anaphase of Pisang Lilan.
FIGURE X.—Second metaphase of Long Tavoy with supernumerary spindle.
FIGURE Y.—Second anaphase B6. Note lagging bivalent.
FIGURE Z.—Six cell "tetrad" of Pisang Lilan.

TaNiPa) may be considered as being completely normal meiotically. The fourth (labelled Philippine unidentified) tends to have one asynaptic chromosome pair in about half the first metaphase plates. In the majority of cells segregation occurs without chromosome loss.

Musa Nagensium

The two varieties of this species used in the present study are probably identical, the different labels representing only different importations of the same thing. In any event they both behave as normal diploids.

DISCUSSION

Of the 18 diploid clones examined, only three showed extensive meiotic aberrations, but seven others representing all four species did show occasional abnormalities at some stage. Most of these aberrations are apparently associated with faulty pairing usually of one chromosome pair. In Zebrina E and Philippine unidentified the tendency to asynapsis is marked. In the latter variety no more than a single pair seems to be involved, but in the former up to seven pairs may be affected. Whether this faulty pairing represents "hybridity," the presence of "asynaptic genes," or accident due to atypical environment is a question that can be answered only through fairly extensive cyto-genetical investigations. The variation both within and between the three ZE navels must almost certainly be ascribed to factors within the third category, but such marked susceptibility to environmental control may be indicative of either of the other two possibilities.

The presence of a trivalent in half the first metaphase plates of Pisang Lilan is definitely indicative of structural hybridity—presumably a reciprocal translocation involving most of a chromosome arm.

With respect to breeding behavior, the diploid clones so far used, including Pisang Lilan, must be considered as generally giving haploid pollen. Tetraploid offspring from Gros Michel×*M. acuminata* varieties must therefore contain three sets of Gros Michel and one set of *M. acuminata* chromosomes, a further indication of the soundness of the "restitution nuclei" hypothesis for Gros Michel.

Some indication of the origin of Gros Michel is given by the fact that only members of the *M. acuminata* group have been successfully crossed with it. The implication, of course, is that the two are closely related. Further evidence on this point will be discussed in the third paper of this series.

Information concerning chromosome morphology is largely negative in so far as comparative value is concerned. It is quite impossible to distinguish between varieties on the basis of chromosome appearance. Even within varieties the 11 bivalents are so similar that they cannot be individually distinguished except for two relatively long bivalents and one very small one (see fig. A–R). The rest are almost identical in appearance.

SUMMARY

Studies of meiosis in 18 diploid clones have been carried out.

These clones represent four species—namely, *Musa acuminata*, *M. rubra* (?), *M. Balbisiana*, and *M. Nagensium*.

These varieties are considered to be normal diploids, with the exception of ZE (*M. acuminata*) and Philippine unidentified (*M. Balbisiana*), which tend to asynapsis of one or more chromosome pairs, and Pisang Lilan (*M. acuminata*), which is a reciprocal interchange hybrid.

The hypothesis is upheld that the tetraploid offspring of Gros Michel × *M. acuminata* varieties result from triploid gametes formed by Gros Michel through suppression of the first division of meiosis combined with haploid gametes from *M. acuminata*.

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CYTOLOGICAL STUDIES IN THE MUSAE. III. MEIOSIS IN SOME SEEDLING CLONES

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INTRODUCTION

THE two previous papers of this series (WILSON 1946a, 1946b) dealt with meiosis in several representative clones of triploid and diploid Musae. As a continuation of these studies, the present paper is largely concerned with the general behavior of the chromosomes during meiosis in certain offspring of triploid-diploid crosses. The female parent in every case was either Gros Michel or one of its "sports," Highgate, while the male parents were several varieties of the species *Musa acuminata* Colla. The general cross, Gros Michel×*M. acuminata*, yields very few seeds as expected, and most of those that prove viable are tetraploids (2n=44). Occasional aneuploids, heptaploids, and a few diploids are also produced (CHEESMAN and LARTER 1935; LARTER 1935 1936–9, and unpublished; CHEESMAN and DODDS 1942). Aneuploids are rarely vigorous and generally die before maturity. Heptaploids are usually thick-leafed, slow growing dwarfs which seldom if ever flower. Of the four diploids so far discovered, three are vigorous and have flowered at least once, while the fourth lived for several years without flowering and has now died out.

In so far as the diploid and tetraploid seedlings are concerned, the present investigation is largely concerned with the question of degree and type of first metaphase pairing.

MATERIALS AND METHODS

Tetraploid seedlings representing the four families, Gros Michel×Zebrina "A" (J×ZA); Gros Michel×Calcutta 4 (J×C_4), Gros Michel×B_7 (J×B_7), and Gros Michel×Pisang Lilan (J×PL) were used as well as the diploid seedlings 349 (H×K), 5387 (J×C_4), and 5445 (H×ZA).

Methods of preparation were as previously described (WILSON 1946a).

OBSERVATIONS

I. Tetraploids

a. First metaphase pairing

In general all four families show relatively complete pairing (fig. A–D), the commonest formula being 14II, 4IV. In most cases where univalents are present they appear to be associated with an equal number of trivalents, and the number of quadrivalents is proportionately reduced, an indication that the chromosomes which go to form trivalents are the same as those generally forming quadrivalents. The quadrivalent frequency is variable, however, and all families have shown up to seven (table 1).

The three families J×ZA, J×B_7, and J×PL show only slight and doubtfully

significant differences in pairing relationships. The fourth family J×C4, however, tends to have 5IV oftener than four and to show an increase over the other families in trivalent frequency. Owing to the difficulty of analysing more than a few cells in each family in detail, most of the figures given are based on estimates carried out as follows: in a fair proportion of first metaphase cells the total number of associations can be counted and univalents are generally well defined. On the assumption that each univalent represents a corresponding trivalent (an assumption borne out by clear cases), a fair estimate of the cell

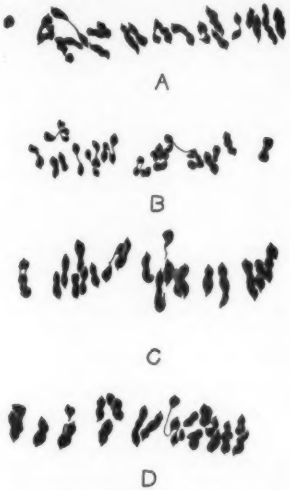

FIGURE A.—First metaphase from S5065 (J×ZA) with 2I, 14II, 2III, 4IV.
FIGURE B.—First metaphase from S6098 (J×C4) with 14II, 4IV.
FIGURE C.—First metaphase from S5455 (J×B7) with 14II, 4IV.
FIGURE D.—First metaphase from S5411 (J×PL) with 14II, 4IV.

formula can be made. By checking this estimate against such parts of the cell as can be clearly analysed the error is reduced to a minimum. Such small differences as appear to exist between the three most similar families are considered to be of doubtful significance. The differences between J×C4 and the other families, however, are of a degree and constancy sufficient to warrant the opinion that they are real.

b. Chromosome segregation

Counts of first anaphase, second metaphase, and second anaphase indicate that between one-fifth and one-third of all pollen grains receive 22 chromosomes (table 2), but such gametes are, of course, not necessarily balanced. On the average about one chromosome is lost through lagging or precocious separation. Unequal segregation is also common (fig. E). Second anaphase

TABLE 1

Frequency of multivalents in tetraploid seedlings.

CROSS AND SEEDLING NUMBER		NUMBER OF QUADRIVALENTS									SUM OF TRIVALENTS AND QUADRIVALENTS									
		0	1	2	3	4	5	6	7	8	0	1	2	3	4	5	6	7	8	9	10
J×ZA	5065			2	2	10	1			1			1	1	12	1			1		
	5433	2		2	3	3					1		2	1	7						
	5443						1									1					
	6196		1	2		1	1					1	—	—	2	2					
	6197				2	2	4							1	2	5					
	6201	1	2	3	15	10					1	2	2	7	19						
	Total	3	3	9	22	26	7	—	—	1	2	3	5	10	42	9	0	0	1		
J×C4	5376	1	1	1	—	1	1					1	1	—	1	—	1				
	5378	1	3	1	4	1	2		1			1	—	4	3	3	—	—	—	2	
	5382						1			1							1	—	—	1	
	5469				2	3	5	2	3	3				1	—	3	2	8	1	2	1
	6098		1	2	3	6	3	4	2				2	2	2	7	5	1	2	—	—
	Total	2	5	4	9	11	12	6	6	4	0	2	3	7	6	13	9	9	3	5	1
J×B7	5033		2	2	8	3	3	1	1	1				5	6	1	6	—	1	1	1
	5040				6	3								1	5	3					
	5370			5	2	6	1							1	10	3					
	5451				3	4	1								7	—	1				
	5455					2									2						
	Total	0	2	7	19	18	5	1	1	1	0	0	0	7	30	7	7	0	1	1	1
J×PL	5055					1									1						
	5056			1	1									1	1						
	5411				2	4								1	5						
	5420					2									2						
	6204				2	2								2	2						
	6206					4	—	2							4	—	2				
	6217	2	1	—	1	3	1	—	1		2			1	2	3	—	1			
	Total	2	1	1	6	16	1	2	1	0	2	0	0	5	17	3	2	1			

separation is generally normal (fig. G). "Omitted" chromosomes usually divide to give two micronuclei (fig. F, G, H).

c. Tetrads

The number of micro-cells in addition to the usual four macro-cells of the tetrads ranges from zero to six with the zero and two groups forming the majority (table 3). Odd numbers of micro-cells are unusual, which suggests that these cells are formed by division of lagging or "omitted" chromosomes. This view is also supported by the fact that the micro-cells occur as a rule in closely associated pairs with the two members of a pair being very similar in size and shape (fig. H). The variation within is only slightly less than between families and of doubtful significance. On the whole, the six-cell tetrads seem to be the most frequent with the four-cell ones second, although the two are about equal in the family J×ZA. It is probable that the two micro-cells of the six-cell tetrads are generally formed from a single lagging chromosome which agrees with the average loss during chromosome segregation (table 2).

d. Pollen grain diameters

The pollen grain diameters as expected are on the average somewhat greater in the tetraploids than in the triploids; 70–75 as compared to 60–70 (table 4).

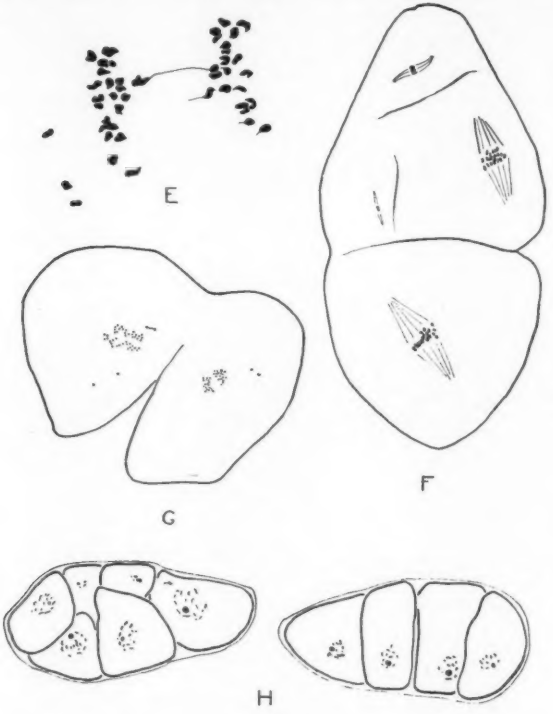

FIGURE E.—First anaphase from S5451 (J×B7) Formula 3+21−20.

FIGURE F.—Second metaphase from S5376 (J×C4) Formula 3/1/20/20.

FIGURE G.—Second anaphase from S5420 (J×PL) Formula 1−1+1−1+22−22/19−19 +1−1.

FIGURES H.—Four and six cell tetrads from J×C4.

Likewise, as expected from the more normal segregation, the range of size values is not as great.

II. Diploid seedlings

The three diploid seedlings studied proved to be as normal in chromosome pairing and segregation as the naturally occurring diploids described in the previous paper (WILSON 1946b) (see table 5). Indeed one of them, 5387, showed no aberrations of any kind. The other two, 349 and 5445, showed a few cases of asynaptic pairs at first metaphase and occasional lagging and mis-

TABLE 2

Chromosome segregation.

CROSS AND SEEDLING NUMBER		NUMBER OF CHROMOSOMES PER POLE															MEAN NUMBER LOST OR LAGGING
		13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
J×ZA	5064							1	5	9	10	1	3				
	5065					1	—	1	1	6	3	2					
	5433						2	2	2	—	4	1	1				
	5443						1	1	2	9	16	9	5	1			
	6196									1	2	1					
	6197								3	5	8	2	1				
	6200					1	1	—	5	6	7	3	3				
	6201					2	2	6	12	9	11	10					
	Total					4	6	11	30	45	61	29	13	1			0.6
J×C4	5376				2	—	5	6	13	16	18	11	3	1	—	1	
	5378	1	—	1	—	1	3	7	12	11	21	6	2				
	5382					1	1	—	6	8	8	1	1				
	5469				1	1	5	2	10	6	3	3	2				
	6098							10	14	11	13	4	4				
	6228						1	1	1	4	10	3	—	1			
	Total	1	—	1	3	3	15	26	56	56	73	28	12	2	—	1	1.1
J×B7	5033							2	5	10	4	1					
	5040							3	—	11	3	2					
	5370						1	—	6	3	8	1	1				
	5451					1	2	3	5	6	6	—	2				
	5455						1	5	22	16	14	1					
	Total					1	4	13	38	46	35	5	3				1.2
J×PL	5055								2	5	4	2					
	5056								9	9	11	3	2				
	5411								6	15	3	6					
	5420					1	—	—	10	12	6	6	3				
	6204		1	—	—	—	—	—	3	6	2	5	3				
	6206				1	—	—	1	1	—	1						
	6217				1	2	3	1	4	5	4	1					
	Total		1	—	2	3	3	2	35	52	31	23	8				0.9

division at first anaphase. Also both had pollen grain diameters greater than the diploid average.

Seedling 349 is known to be highly fertile and is definitely hybrid since seeds obtained by selfing give rise to a segregating population. The other two seedlings have not yet been tested for fertility, but both give abundant, apparently normal pollen.

DISCUSSION

Pairing in the tetraploid offspring of Gros Michel×*M. acuminata* varieties is on the whole complete, which means that among the three Gros Michel chromosome sets there is at least one which is reasonably homologous with the chromosomes of the several varieties of *M. acuminata* used. This set of Gros Michel chromosomes can therefore be labelled "acuminata type." The very frequent appearance of 4IV also suggests that at least four chromosomes in

each of the remaining two sets are also "acuminata." Furthermore, the fact that up to 8IV may be formed suggests that at least eight of the 11 chromosomes in these two sets are semi-homologous with "acuminata." The pairing relationships in the triploid Gros Michel itself indicated that two of the three sets were nearly strictly homologous, while the third was semi-

TABLE 3
Cells per "tetrad."

CROSS AND SEEDLING NUMBER		NO. TETS.	NO. CELLS PER "TETRAD"			
			4	6	8	10
J×ZA	5064	100	43	48	9	
	5065	100	45	45	10	
	5433	100	52	44	3	1
	5445	100	34	52	12	2
	6200	100	46	43	11	
	Total	500	220	232	45	3
J×C4	5047	100	32	59	9	—
	5376	100	23	63	14	—
	5378	100	47	50	3	
	6224	100	17	65	16	2
	6225	100	22	69	9	
	Total	500	141	306	51	2
J×B7	5033	100	21	69	8	2
	5040	100	25	57	16	2
	5370	100	21	66	12	1
	5451	100	11	65	22	2
	5455	100	14	64	20	2
	Total	500	92	321	78	9
J×PL	5055	100	30	55	13	2
	5056	100	33	55	10	2
	5411	100	39	49	11	1
	5420	100	35	50	14	1
	6204	100	33	50	16	1
	Total	500	170	259	64	7

homologous (WILSON 1946a). The evidence is therefore strongly in favor of the view that all three chromosome sets of Gros Michel are or originally were "acuminata types" or closely related thereto. The complete pairing in the diploid seedlings discussed may also be considered as strong support for this supposition, since it is unlikely that exactly the same set of 11 Gros Michel chromosomes occurs in each of them. It seems reasonable to conclude, therefore, that Gros Michel is closely related to the *M. acuminata* group in origin at least.

Improving the diploid parent: The logical method of breeding for resistance to disease is to cross the best available commercial type with the best resistant type and back cross to the former. In the present case the best commercial type is the Panama disease and leaf spot susceptible Gros Michel and the best available resistant types, having an eye to the importance of fertility are the wild bananas. Theoretically, Gros Michel should be used as the female parent and the more fertile wild types as the male parent—that is, the primary cross should be Gros Michel×wild type followed by the back cross Gros Michel ×(G.M.×wild). This technique however, is not satisfactory. The primary cross generally gives a tetraploid and the appropriate back cross a pentaploid

FIGURES I to K.—First metaphase chromosomes from the diploid seedlings 349, 5387, and 5445, respectively.

which experience has shown tends to be a thick leafed, slow growing dwarf which seldom if ever flowers. The policy of searching among the primary tetraploids has therefore been established (Larter 1937–9). A fair proportion of such primary tetraploids have many of the desired features—for example, immunity to Panama disease and leaf spot, good flavor and color, high "grade" and compactness of bunch. The best of them, however, tend to be rather short fingered. Since on the current theory of gamete formation in Gros Michel the tetraploid must carry the entire Gros Michel complex, the trouble must lie with the diploid parents used. These are all much shorter fingered than Gros Michel so that the problem is to develop a long fingered male parent without, of course, sacrificing other desirable characteristics.

This question has been discussed by DODDS (1943) who suggests the derivation of a "synthetic diploid" from secondary triploids. A somewhat similar suggestion is the derivation of a diploid from certain long fingered plantain like F_2 tetraploids (LARTER unpublished) as shown in table 6. The finding of the diploid seedlings discussed above with their apparently normal meiosis suggests a simple alternative. In theory such seedlings contain a single set of Gros Michel chromosomes plus a set of acuminata chromosomes. If these seedlings are resistant to Panama disease (and 349 is definitely so), then it should be possible to obtain among the segregants from inbreeding a form which contains nearly "pure" Gros Michel genes plus the factors for immunity. Further crossing between the primary seedlings or their more promising segregants

TABLE 4

Pollen grain diameters.

CROSS AND SEEDLING NUMBER		NUMBER MEASURED	DIAMETER	STANDARD ERROR
J×ZA	5064	100	69.9	.55
	5065	100	72.4	.55
	5433	100	74.1	.49
	5443	100	77.2	.63
	6200	100	73.8	.49
	Total	500	367.4	
	Mean		73.5	
J×C4	5376	100	73.5	.76
	5378	100	76.7	.72
	5382	100	77.4	.84
	5469	100	75.7	.71
	6098	100	74.4	.61
	Total	500	377.7	
	Mean		75.5	
J×B7	5033	100	75.3	.60
	5040	100	73.6	.58
	5370	100	73.8	.64
	5451	100	72.8	.66
	5455	100	75.9	.52
	Total	500	371.4	
	Mean		74.3	
J×PL	5411	100	72.6	.59
	5420	100	72.1	.54
	6204	100	75.0	.60
	6206	100	69.6	.77
	6217	100	72.8	.66
	Total	500	362.1	
	Mean		72.4	

should further increase the chances of accumulation of suitable genes. Crosses involving this technique have recently been initiated, but it must be pointed out that there is no certainty that any or all three of the seedlings together contain all the appropriate genes. In any event, between 1000 and 2000 primary segregants must be raised before even the initial potentialities can be assessed.

TABLE 5

VARIETY	FIRST METAPHASE		FIRST ANAPHASE		SECOND METAPHASE		SECOND ANAPHASE		NO. CELLS PER "TETRAD"						POLLEN GRAIN DIAMETER
	NORM.	OTHER	NORM.	OTHER	NORM.	OTHER	NORM.	OTHER	3	4	5	6	7	8	
349 (H×K)	29	2 (10II+2I)	8	2 (12–10)	13	2 (10+1/11) 1 (10/12)	15	1 (9–9/13–13)	1	463	30	32	—	—	71.4±.44
5387 (J×C4)	34	0	17	0	20	0	12	0	—	100	—	—	—	—	58.5±.31
5445 (H×ZA)	44	6 (10II+2I)	12	2 (10–2–10) 1 (10–4–8)	24	1 (12/10) 1 (11/1/10)	9	0	—	97	1	1	—	1	67.8±.28
ZA	28	0	7	1 (10–2–10)	10	1 (10/2+10)	9	0	—	100	—	—	—	—	58.7±.34

H = Highgate variety of Gros Michel
J = Gros Michel (Jamaica Banana)
K = *Musa kewensis* a variety of *M. acuminata*
C_4 = Calcutta 4 also a variety of *M. acuminata*
ZA = Zebrina Clone "A" a variety of *M. acuminata*

GENERAL SUMMARY

During the course of three papers the behavior of the chromosomes in microsporogenesis of diploid, triploid, and tetraploid Musae has been discussed. Attention has been particularly centered on those forms used in breeding—namely, the triploid Gros Michel, the diploid varieties of *Musa acuminata*, and the tetraploid and diploid offspring of Gros Michel—*M. acuminata* crosses.

The fact that the bulk of the viable offspring of the general cross Gros Michel × *M. acuminata* are tetraploids plus the fact that all the *acuminata* varieties used are relatively normal and expected to give haploid gametes support the assumption that most of the viable eggs of Gros Michel are triploid. Such triploid eggs are presumably produced through the formation of restitution nuclei during microsporogenesis (LARTER unpublished). Some direct evidence in support of this hypothesis has been noted in the tendency for at least partial suppression of the first division of meiosis (see also DODDS

TABLE 6

F_2(2n=44)	×		2n=22 (long fingered type—for example, Pisang Lilan).
	↓		
	*2n=33	×	2n=22
		↓	
		2n=22	(Long fingered. Immune to Panama disease.)

* It is known that such triploids do give a reasonable number of haploid gametes.

and PITTENDRIGH 1946). The chief difficulty with this hypothesis is the segregation that occurs among F_1 tetraploids (LARTER unpublished). If all the Gros Michel chromosomes are present in the tetraploid offspring, segregation must come from the diploid male parent, but in so far as the characters concerned have been studied (especially disease resistance) the male parents used appear to be homozygous. One possible explanation, however, is that the wild types do segregate for some physiological or morphological factors predisposing to disease but are not themselves affected.

The relatively complete pairing in the tetraploid seedlings gives some indication of close relationship between Gros Michel and the *acuminata* group The general conclusion based on the first metaphase associations of both Gros Michel and its tetraploid offspring as well as the highly normal pairing in the three diploid seedlings is that all three chromosome sets of Gros Michel are "*acuminata* type." This in a cytological sense would make Gros Michel essentially a triploid form of *M. acuminata*. Genetically, of course, it may differ widely from any synthetic triploid derived from the present *M. acuminata* group, since selection for desirable commercial characteristics has been carried on for several thousands of years.

The potential value of the diploid seedlings resulting from Gros Michel × *M. acuminata* varieties in the synthesis of a more suitable male parent has been discussed.

ACKNOWLEDGMENT

The author wishes to take this opportunity of expressing his indebtedness to MR. L. N. H. LARTER, Senior Botanist for Jamaica, for supplying him with materials and unpublished information as well as for many valuable discussions of data.

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GENETIC AND NONGENETIC SOURCES OF VARIATION IN THE WEIGHT RESPONSE OF THE IMMATURE RAT OVARY TO A GONADOTROPHIC HORMONE[1]

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INTRODUCTION

A COMMON method of bioassay of gonad-stimulating preparations is based on the gain in the ovary-weight of the immature female rat in relation to the amount of injected material. The large variability in the response of the ovaries of different rats of the same age to similar treatment is common knowledge of endocrinologists. The exact sources of this variability and their relative importance, however, are not common knowledge, even though some of them, such as strain and body weight, are sometimes considered in designing experiments or comparing experimental results.

The results reported here indicate the extent to which some of the factors influence this variability of stimulated ovary weights in a particular colony of rats. They stress the need for cognizance of these elements of variability in designing experiments to gain more efficient use of animals and to interpret results of experiments with more precision.

MATERIALS AND METHODS

The albino rats used in this work are descendants of Sprague-Dawley stock introduced into this laboratory May 1940. The conditions under which they have been maintained have been as uniform as practically possible (unavoidable changes in feed, temperature, animal caretakers, etc., occurred from time to time).

There has been a gradual increase in inbreeding in about half the colony and practically none in the rest. Selection has been directed toward improving size of litter at birth and body weight at six weeks and 13 weeks of age and recently to developing inbred and outbred lines of rats with ovaries highly refractory to the hormone in some lines and highly responsive in others. Other planned deviations from natural selection and random mating have been an early elimination of sickly rats and an attempt to breed each female at least twice to the same male and discard her at the weaning of her fourth litter or earlier if persistently infertile.

The size of the litter was reduced or increased to six rats on the day following birth. A litter larger than six at birth was reduced to six by approaching a ratio of five females to one male as closely as possible. For a litter smaller in number than six, excess marked young from litters born in the same day were

[1] Paper from the Department of Genetics, No. (354), Agricultural Experiment Station, Univ. of Wisconsin. An abstract has been published (Genetics **30**: 2–3, 1945) of a report prepared for the 1944 meetings of the Genetics Society.

transferred whenever possible until a litter size of six was reached. When there were as many as four females in each of at least two contemporary litters, reciprocal transfers of some of the female young were made between the litters on the day following birth. This procedure allowed (at 25 days of age) contemporary characterization of ovarian response of a dam's own young raised by herself, her own young raised by another dam, as well as another dam's young raised by her. These reciprocal transfer data provide information on maternal effects from conception to birth and from birth to weaning.

At three weeks of age the young were weaned and the dam rebred. The young were marked individually; the males were kept for a later selection on body weight and one female was taken at random from each litter for later use in the breeding colony. The other females were injected subcutaneously with a standard dose of gonadotrophic hormone[2] for three days, beginning on the 21st day of age, and then autopsied approximately 96 hours later. An attempt was made to standardize the dosage at a level which would produce an average ovary weight of 65 mg in Sprague-Dawley rats selected at random from 21-day old females weighing 40–45 gm.

This response (65 mg) was defined as a rat ovarian unit by CARTLAND and NELSON (1938) because it had been found to be the approximate midpoint of the steepest linear part of the ovary-weight-dosage curve of pregnant-mare-serum assays. They point out that this is "a region of high sensitivity to small changes in dosage and thus constitutes the most suitable part of the ovarian curve for the quantitative assay of this hormone."

The date, individual rat number, post-mortem body weight (B.W.), and weight of the ovaries (O.W.) were noted at autopsy. The ovaries were freed of the encapsulating bursae before they were weighed to the nearest milligram on a torsion balance.

ANALYSIS OF DATA

The total number of autopsied individuals involved in this report, which covers the period from July 1940 to December 1942, is 3,257. The mean O.W. is 67.5 mg, with a mean square of 822 and standard deviation of 28.7. The frequency histogram of O.W. is given in figure 1.

Influence of "Month"

There was no obvious association between O.W. and any seasonal classification that was made but there were obvious long time and large fluctuations throughout the two and one-half year period. An arbitrary classification was made on the basis of calendar month, which was the shortest interval of time that would give appreciable numbers per class—never fewer than sixty (average = 112).

[2] The hormone, pregnant mare serum, was generously provided by UPJOHN & Co., Kalamazoo, Michigan; DR. W. H. MCSHAN, Department of Zoology, UNIVERSITY OF WISCONSIN, kindly prepared relatively pure gonadotrophic preparations of pregnant mare serum in dry form by the method of CARTLAND and NELSON (1937). These preparations were stored in a dessicator until the time of injection, when enough for a three- or four-day period was dissolved in distilled water and kept at about 5°C over the three- or four-day period in which it was being injected.

Analysis of variance (table 1) of ovary weights classified on this basis indicated a highly significant difference between months. These differences accounted for about 17 percent of the O.W. variability (intra-class correlation = .17) and are interpreted as being due to nongenetic factors. A similar

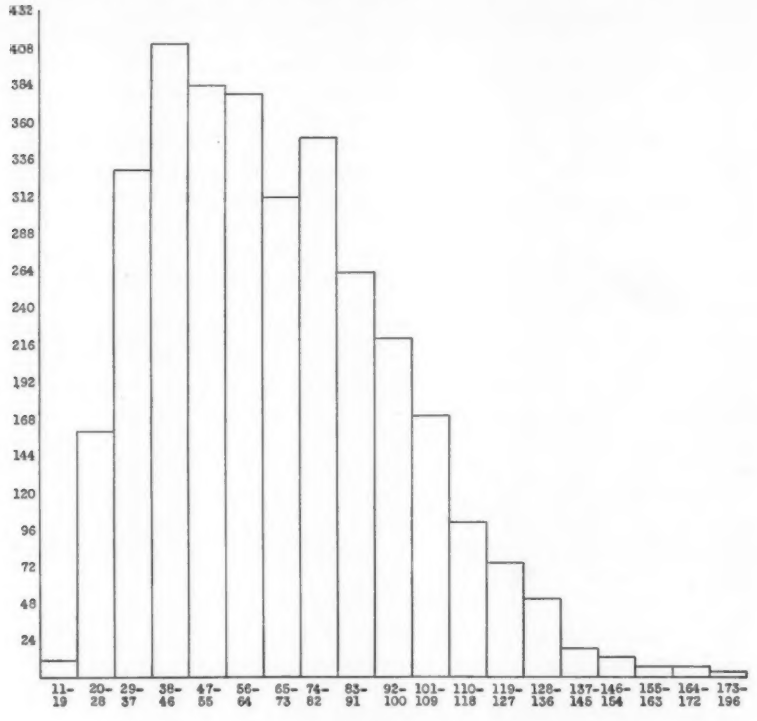

FIG. 1.—Frequency histogram of ovary weights.

monthly effect (intra-month correlation = .25) was noted for post-mortem body weight (table 1)—the variable discussed next.

Influence of Post-Mortem Body Weight

The practice of using rats of a specified age and within a definite weight range for certain endocrine work suggested an association between B.W. and O.W.

Analysis of covariance between B.W. and O.W. (classified on the basis of month) bore out this contention by showing a highly significant negative association between these two variables not only within months but also between the averages for months (table 2). Hence part (.39 = between month r^2) of the between-month variance in O.W. is due to B.W. variation.

The within-month regressions of O.W. and B.W. were tested for homogeneity. They did not appear constant from month to month ($P \cong .05$). Within

TABLE 1

Analyses of variance in ovary weight and post-mortem body weight showing the effect of "month."

SOURCE OF VARIATION	D/f	MEAN SQUARES		INTRA-MONTH CORRELATIONS	
		OVARY WEIGHT	BODY WEIGHT	OVARY WEIGHT	BODY WEIGHT
Total	3256	822	64		
Between months	28	15039	1689		
Within months	3228	699	49	.17**	.25**

** $P < .01$.

the ranges of B.W. and O.W. found here they were negative in all cases except one in which it was insignificantly positive (-2.287 to $+0.6929$).

Adjustment for "Month" and Post-Mortem Body Weight

The present selection program in this colony is directed toward bringing about divergence between lines on the basis of ovarian sensitivity while main-

TABLE 2

Analysis of covariance in ovary weight and post-mortem body weight showing the effect of "month."

SOURCE OF COVARIATION	D/f	SUMS OF PRODUCTS	REGRESSION COEFFICIENTS	CORRELATION COEFFICIENTS
Total	3256	−329,315	−1.59	−.44**
Between months	28	−88,409	−1.87	−.63**
Within months	3228	−240,906	−1.51	−.40**

** $P < .01$.

taining similarity between the lines in other respects. Inasmuch as there is an association between B.W. and O.W., there is a need for equalization of that part of O.W. variation which is due to B.W. fluctuations to avoid probable divergence between lines in body weight. The change in the B.W.-O.W. regression from month to month led to an intra-month correction of O.W. for B.W. by means of a simple regression equation. The mean O.W.-B.W. coordinates for the month are taken as the standard to which the O.W.'s are adjusted.

In order to eliminate some of those long-time, less tangible factors mentioned under "month," these B.W.-corrected O.W.'s were next adjusted to an over-all monthly standard which was taken to be an O.W. of 66 mg found with a B.W. of 45 gm on the basis of the averages of the first 18 months' data.

These adjustments allow for the combining of information from rats of varying sizes and birth dates, thereby facilitating the analyses necessary for isolating the other sources of variation and providing a more logical O.W. basis upon which selection can be practiced month by month.

The results reported below are based on these corrected ovary weights (C.O.W.) and should therefore be interpreted as the results which would have been obtained if all these records had been secured within an average month on rats weighing about 45 gm at autopsy.

Influence of Heredity and Other Factors

Subdivision of the variation remaining in O.W. after standardization for B. W. and monthly fluctuations was done by using WRIGHT'S (1921, 1934) method of path coefficients to analyze certain simple correlation coefficients (table 3) into their component parts.

The correlation between full sister litter mates (0.39 ± 0.04) is statistically highly significant ($P < .01$). It may have resulted from the action of any one or any combination of the factors listed under "Possible biological constituents" in table 3.

The expression of the genes common to full sister litter mates may be responsible for all or part of their phenotypic covariation. Full sister covariation has been analyzed by WRIGHT (1935) into that portion which acts as though it were based on additive genes and into another portion which deviates from such a scheme in the manner expected when dealing with non-additive allelic gene action (dominance) and non-additive non-allelic gene action (epistacy). On the average, a correlation of 0.50 between full sisters exists for those characteristics whose variability conforms wholly to the additive scheme ($\frac{1}{2}g^2$).

According to WRIGHT (1935), in a rather wide variety of cases the correlation between full sibs for that part of the variation which is due to dominance and epistatic deviations from the additive scheme is 0.25 for each of them ($\frac{1}{4}d^2$ and $\frac{1}{4}e^2$). On this assumption, then, only one-fourth of the dominance deviations and one-fourth of the epistatic deviations causing variation for this particular character in this population would have contributed to the phenotypic similarity of full sisters. The exact contributions of dominance deviations and epistatic deviations to the observed C.O.W. correlations between various relatives however, is uncertain. The uncertainty arises from the fact that C.O.W. may not be a character which conforms to the assumptions basic to WRIGHT'S derivation, or, if it should be, there is still some uncertainty as to the contribution made by second and higher order gene interactions to the correlations between various relatives. The approximate portions of the dominance and epistatic deviations which would be expected to contribute to the correlations under those assumptions are given in table 3.

In addition to similarity between sisters which may arise from common heredity, there may also be similarity due to the common maternal influences (p_1^2, p_2^2, t_1^2, and t_2^2) from conception to the time of autopsy, and likewise to contemporaneous nongenetic, nonmaternal factors (c^2) operating during this same interval. Subdivision of the variation contributed by the common

TABLE 3

Correlation coefficients and their biological interpretation for ovary weights standardized for autopsy body weight of the individual and monthly fluctuations.

PAIRING OF INDIVIDUALS	NUMBER OF PAIRS	POSSIBLE BIOLOGICAL CONSTITUENTS*	CORRELATION COEFFICIENTS: OBSERVED	CORRELATION COEFFICIENTS: ASSUMING: $g^2=.36$, $d^2=.04$, $t_2^2=.09$, $c^2=.13$, $a^2=.38$, $e^2=t_1^2=p_1^2=p_2^2=0$
Full Sisters				
(1) Litter mates	418	$\frac{1}{2}g^2+\frac{1}{4}d^2+\frac{1}{4}e^2+p_1^2+p_2^2+t_1^2+t_2^2+c^2$ =	+0.39±0.04	.41
(2) Nonlitter mates	223	$\frac{1}{2}g^2+\frac{1}{4}d^2+\frac{1}{4}e^2+p_1^2+p_2^2$ =	+0.23±0.06	.19
(3) Transfer and nontransfer (contemporary)	314	$\frac{1}{2}g^2+\frac{1}{4}d^2+\frac{1}{4}e^2+p_1^2+t_1^2+c^2$ =	+0.33±0.05	.32
(4) Transfer and nontransfer (noncontemporary)	134	$\frac{1}{2}g^2+\frac{1}{4}d^2+\frac{1}{4}e^2+p_1^2$ =	+0.19±0.08	.19
Half Sisters				
(5) Paternal	952	$\frac{1}{4}g^2+\frac{1}{16}e^2$ =	+0.09±0.03	.09
(6) Maternal	700	$\frac{1}{4}g^2+\frac{1}{16}e^2+p_1^2+p_2^2$ =	+0.06±0.04	.09
(7) All	1652	$\frac{1}{4}g^2+\frac{1}{16}e^2$ =	+0.08±0.02	.09
Aunts-Nieces				
(8) Paternal	359	$\frac{1}{8}g^2+\frac{1}{16}e^2$ =	+0.10±0.05	.09
(9) Maternal	889	$\frac{1}{8}g^2+\frac{1}{16}e^2$ =	+0.10±0.03	.09
(10) All	1248	$\frac{1}{8}g^2+\frac{1}{16}e^2$ =	+0.10±0.03	.09
Half Sisters and Aunt-Nieces				
(11) (7)+(10)	2900	$\frac{1}{8}g^2+\frac{1}{16}e^2$ =	+0.09±0.03	.09
Unrelated Reared By Same Dam				
(12) Contemporary	509	$p_1^2+t_2^2+c^2$ =	+0.24±0.04	.22
(13) Noncontemporary	581	p_1^2 =	−0.05±0.04	.00
Unrelated Reared By Different Dam				
(14) Contemporary	342	c^2 =	+0.13±0.06	.13
(15) Complete Determination = $g^2+d^2+e^2+p_1^2+p_2^2+t_1^2+t_2^2+c^2+a^2=1.00$				

* Fraction of Variance Due to:

Additive gene effects.....g^2
Dominance deviations from additive scheme.....d^2
Epistatic deviations from additive scheme.....e^2
Permanent maternal prenatal factors.....p_1^2
Permanent maternal postnatal factors.....p_1^2
Temporary maternal prenatal factors.....t_1^2
Temporary maternal postnatal factors.....t_2^2
Contemporary nongenetic, nonmaternal factors.....c^2
Accidents of development or technique.....a^2

maternal factors has been made arbitrarily on the basis of whether they are operative prenatally (p_1^2, t_1^2) or postnatally (p_2^2, t_2^2), and whether they act as maternal influences which are permanent (p_1^2, p_2^2) in the sense of creating similarity between individuals born at different times or merely effective in a particular prenatal (t_1^2) and postnatal (t_2^2) period.

The constituent fractions of the variance given for correlations in other pairings of individuals (table 3) indicate symbolically the possible causative com-

ponents of those associations. The only correlation coefficient which is clearly not statistically significant is that between unrelated non-contemporary individuals reared by the same dam from birth to weaning (equation 13, table 3). The maternal half sister correlation (eq. 6) would not be considered statistically significant if it stood alone ($P \cong .10$), but it does not differ significantly from the significant paternal half sister correlation (eq. 5) nor from the significant aunt-niece correlations (eq. 8, 9 10), and all of these on genetic grounds should be similar, excluding major sex-linked genes. The standard errors are attached to the correlation coefficients and should serve as a constant reminder of the fiducial limits of the coefficients in the calculations which follow.

The squared path coefficients (portions of the variance) are simply additive. This means that solution of these equations for their component variables can be readily accomplished by taking a series of differences and making successive approximations until a good fit is secured for the series of equations involved.

The most direct estimate of heritability (additive gene effects) in data of this kind can be secured from the half sister (eq. 5, 6, 7) and aunt-niece correlations (eq. 8, 9, 10). There is, as implied above, no significant difference between the paternal and maternal aunt-niece correlations, hence no indication of an effect of sex-linked genes on C.O.W. variation. It was also noted above that there is no significant difference between the correlation coefficients for paternal and maternal half sisters. This, together with the insignificant difference between the maternal half sister correlation and the two aunt-niece correlations points to the practical unimportance of the contribution, if any, of permanent maternal effects, whether of prenatal (p_1^2) or postnatal (p_2^2) origin.

Because the genetic contributions to the two half sister and two aunt-niece correlations are theoretically the same and no nongenetic factors are obviously involved in either association, no biological reason is evident to prevent pooling the four coefficients which are not significantly different, statistically. The combined value, based on 29 hundred pairs of observations, is 0.09 (eq. 11). This value should theoretically contain one-fourth of the variation due to additive gene effects and an unknown but doubtless small portion of the variation ascribable to nonallelic gene interaction. WRIGHT (1935) has estimated that less than one-sixteenth of the epistatic deviations from the additive scheme would be included in this degree of relationship. This esimate, of course, is only rough, inasmuch as there may be many kinds of gene interactions and these may involve two, three, four, or more gene pairs, with the probability of two related individuals receiving the same combination decreasing as the number of pairs involved increases. Multiplication of this correlation coefficient by four gives an estimate of $g^2 + (\frac{1}{4}e^2) = 0.36$.

What portion of this figure may be assigned to additive gene effects and what to epistatic deviations from the additive scheme? To answer this question, a comparison must be made between the correlation just discussed and those involving full sister relationship.

In order that the four full sister correlations may be combined for use in this estimate, the nongenetic factors must be removed. It has already been stated

that p_1^2 and p_2^2 appear to be insignificant on the basis of the maternal-half-sister versus paternal-half-sister and the maternal-half-sister versus aunt-niece comparisons. Further evidence for the negligible effect of p_2^2 comes from the insignificant correlation between noncontemporary unrelated rats which were reared by the same dam (eq. 13) from birth to weaning. On these figures it is assumed that $p_1^2 = p_2^2 = 0$.

Two estimates of t_2^2, temporary postnatal maternal factors, can now be made. One is by the difference between contemporary unrelated rats reared by the same dam (eq. 12) and contemporary unrelated rats reared by different dams (eq. 14). This gives an estimate of $t_2^2 = 0.11$. Another estimate can be taken from the difference between the coefficients for full sister litter mates (eq. 1) and contemporary full sisters, one of which is reared by her own dam, the other by another dam (eq. 3). This yields $t_2^2 = 0.06$. From these two estimates the value of t_2^2 is assumed to be 0.09. The estimated value for c^2 (0.13 from eq. 14), nongenetic nonmaternal contemporary factors, is given by the correlation between contemporary unrelated rats reared by different dams. The temporary prenatal maternal factors (t_1^2) appear to be insignificant in the light of differences between (eq. 1) and (eq. 2), (eq. 1) and (eq. 4), (eq. 3) and (eq. 2), and (eq. 3) and (eq. 4) after (eq. 1) has had $t_2^2 + c^2$ removed by subtracting (eq. 12), and (eq. 3) has had c^2 removed by subtracting (eq. 14).

The first three terms of the four full sister equations (eq. 1 to 4, inc.) can now be combined to give a pooled estimate of $\frac{1}{2}g^2 + (\frac{1}{4}d^2) + (\frac{1}{4}e^2) = 0.19$. Subtracting twice (eq. 11) from the latter expression gives $(\frac{1}{4}d^2) + (1/8e^2) = 0.01$ or $d^2 + \frac{1}{2}e^2 = 0.04$. If $d^2 = 0$, then $e^2 = 0.08$. On this assumption, $1/16e^2 = 0.005$ and hence (eq. 11) is essentially an estimate of $\frac{1}{4}g^2$, and this leads to a value of 0.36 for additive gene effects. If, on the other hand, it is assumed that $e^2 = 0$, the above conclusion is still valid, but the estimate of d^2 then becomes 0.04. WRIGHT (1931) has concluded that $d^2/g^2 \cong \frac{1}{4}$, and FISHER (1918) that $d^2/g^2 \cong \frac{1}{2}$. In the C.O.W. data these two porportions would lead to $d^2 = 0.09$ and $d^2 = 0.18$, respectively. The fiducial limits of $d^2 = 0.09$ would undoubtedly include 0.04, whereas it is unlikely that 0.18 would. As a first approximation it would appear justifiable to conclude from the dependence of dominance deviations on the additive variance that $d^2 \cong 0.04$ and $e^2 \cong 0$.

The values just presented were substituted in the equations given under "Possible biological constituents" in table 3. The resultant correlation coefficients are given in the right hand column opposite the respective observed coefficients.

DISCUSSION

An estimate has been made in this study of the relative influence of several factors responsible for variation in the ovary weight of immature rats injected with a standard dose of gonadotrophin.

Some of the fluctuations in ovary weight of the test animals from one period of time to another appeared to be nonrandom. These fluctuations were at rather irregular intervals and were not associated with seasons of the year.

During the period covered by these data, changes in quality of feed, reliability of help, type of cages, amount of cage space, batch of hormone, etc., were necessary from time to time. The attempted policy of maintaining relatively standard conditions for long periods of time undoubtedly reduced the variation but did not allow the variation remaining to be assigned readily to its causative factors because of the many intangible factors involved, the confounding of tangible factors, and the lack of information as to how soon after alteration these factors become operative and are reflected in ovarian response.

These arguments led to a classification of ovarian weights on the basis of a time interval which was independent of observations of variation in the data. Calendar month was chosen to satisfy these requirements and because enough observations were made in each month so that it could be well characterized. This somewhat arbitrary classification does not allow of any specific biological interpretation of the 17 percent of the total variation in ovary weight associated with it (intra-month correlation $=0.17$, table 1), but does allow, when this source of variation is eliminated, a combination of data from different months for the analysis which follows and for the selection program more recently undertaken in the colony. This variation (17 percent) can be portioned into that associated with fluctuations in autopsy body weight and that due to other factors. Thirty-nine percent of this monthly variance in ovary weight (between month correlation body weight-ovary weight $=-0.63, r^2=0.39$, table 2) was associated with autopsy body weight. In other words, seven percent of the total variance ($0.17 \times 0.39 = 0.07$, table 4) was associated with the general condition of the rats. The remaining ten percent of the total variance included changes in management which did not influence body weight and intangible or chance factors associated with month.

Autopsy body weight was found to account for 16 percent of the intra-month variance (intra-month $r=-0.40$, $r^2=0.16$, table 2) or 13 per cent of the total ($[1.00-0.17][0.16]=0.13$, table 4). This association was negative within the range of body weights usually encountered. If, however, the body weights were all distinctly below average, there was no evident association between body weight and ovary weight, and the average ovary weights were likewise below average. The explanation for these facts is not known, but it is assumed that in the case of the negative association, the larger the mass into which a given amount of material is injected the less will be the quantity actually reaching the site of stimulation; hence, other things being equal, the smaller will be the response. In the case of the extremely small animals it would appear that their ovaries have not reached a stage of development which allows of the same response as the physically more mature animals.

The influences of other factors on ovarian weight have been estimated on the equivalent of an intra-month, intra-body-weight basis by means of adjusting ovary weight for monthly and intra-month-body-weight fluctuations. This, of course, does not eliminate all of the variation arising from these sources, but it does reduce it to such a degree that the subsequent results can be interpreted as applying to individuals whose ovary weights have been standardized for

month to month variation and to an average body weight of 45 gm—the body weight associated with an average ovary weight of 66 mg. The total variance remaining in the ovary weights after correction for month and body weight was 0.70 of the total variance in the uncorrected ovary weights (1.00−[0.17 +0.13]=0.70, table 4), hence the fractions of the intra-month-intra-body-

TABLE 4

Relative influence of various factors in total variance of ovary weight of immature rats injected with a standard dose of gonadotrophin.

SOURCE OF VARIANCE	FRACTION OF TOTAL VARIANCE	
Inter-month		
Body weight	.07	
Other factors	.10	
		.17
Intra-month body weight		.13
Intra-month-intra-body-weight		
Heredity		
Additive gene effects	.25	
Dominance deviations	.03	
Epistatic deviations	.00	
		.28
Permanent maternal effects		
Prenatal	.00	
Postnatal	.00	
		.00
Temporary maternal effects		
Prenatal	.00	
Postnatal	.06	
		.06
Contemporary nonmaternal factors		.09
Accidents of development or technique		.27
Total		1.00

weight variance in uncorrected ovary weights (table 4) are 0.70 of the fractions of the variance in C.O.W. (table 3).

Heredity of the autopsied individual accounted for about 28 percent of the total variance in ovary weight—40 percent of that within month and body weight. Both of these figures refer to the heredity of C.O.W. and exclude any heritability in ovary weight which may result from its association with body weight, a highly heritable characteristic. The close agreement between the completely independent half sister and aunt-niece correlations, together with the consistency of the full sister correlations, gives strong credence to the esti-

mate of the influence of additive gene effects. This 25 percent of the total variance, 36 percent of intra-month and body-weight variance, may include some of the epistatic deviations from the additive scheme, but on the basis of other calculations it is unlikely that it contains much if any at all. Whether it does or not is immaterial as far as the main predictive value of data of this kind is concerned. That is, the change to be expected in one generation of selection for high or low ovarian sensitivity is dependent on the amount of variance due to additive gene effects plus a part of epistatic deviations, as well as the intensity of selection. Hence, as an estimate of temporary gain it would be valid and probably would not deviate appreciably from the permanent gain—that is, that which is dependent on the simply additive fraction of the variance.

The nonadditive hereditary variance appears to be extremely small. It is impossible, from these data, to be sure of its source. However, it has been ascertained by WRIGHT (1931) and FISHER (1918) that on the assumption of certain theoretical considerations the variance due to dominance deviations bears a certain relation to that due to additive gene effects. The minimum effect that dominance deviations could have on this point of view would be about nine percent. As an approach to this value, all of the nonadditive gene effects are assigned to dominance deviations (three percent of the total variance), leaving none for epistatic deviations from the additive scheme.

There was no evidence of any permanent maternal factors affecting ovarian response. These would include such things as individual peculiarities in uterine environment and in nursing ability, which carried over from one litter to another of the same dam. These are factors which might very well be genetic in the dam even though classified as an environmental influence on the offspring.

Temporary maternal effects were not evident prenatally but were apparently of some importance in the postnatal period. These could have arisen from idiosyncrasies of the dam's uterine environment and nursing ability provided they operated on individual litters rather than affecting all litters of the same dam alike. Such factors might be age of dam, temporary state of health of dam during gestation and when suckling, parasitic or bacterial infection peculiar to particular cages, and any other environmental factors which may be alike for all litter mates but not common to other litters. Postnatal factors of this sort accounted for about six percent of the total variance, nine percent of the intra-month, intra-body-weight variance. These, too, may be due in part to genetic differences between dams in susceptibility to disease or in other physical or physiological differences which are temporary in expression.

In addition to those contemporary factors that are common to litter mates, there may be still other agents of variation which produce likeness between unrelated individuals reared by different dams contemporaneously. These factors accounted for about nine percent of the total variance, 13 percent of intra-month and intra-body-weight variance. Any prenatal and post-natal conditions which affected all contemporary rats alike but distinguished them from other rats on an intra-month and intra-body-weight basis would be included

here—namely, temperature, humidity, length of day, nutrition, parasites, hormone, injection technique, etc., may have operated in such a way.

Finally, 27 percent of the total variance (38 percent of the intra-month-body-weight variance) remains unassigned. This variability is that which is not even common to litter mates and would be expected to remain as the variance between identical twins, for example variability between litter mates in time, place and effectiveness of implantation, securing adequately functioning mammae, bacterial and parasitic infection and any developmental accidents which might differentiate litter mates.

It should be borne in mind that each of these estimates of the relative influence of these factors has fiducial limits which are related to the standard errors of the correlation coefficients from which they were derived. Multiplication of these coefficients increases the standard errors proportionately, and subtraction of one coefficient from another results in a standard error slightly larger than that of each of the components. Offsetting, at least in part, this decrease in precision due to algebraic manipulation is the increased dependability which consistent independent estimates give. It should also be emphasized that these figures pertain only to the population of which this was a representative sample and that in so far as deviations from random mating occur or changes in the management of the colony ensue, these estimates would probably need revision. Likewise, in applying them to other colonies caution should be exercised in accepting them at face value. It is believed, however, that this is a sufficiently representative group of laboratory rats so that these results in general should have rather wide application. In order to decide whether they are applicable to other rats, correlations between rats of the same relationships as those used here could be calculated on smaller numbers and compared with the ones in this paper as a guide to the similarity of trends.

The efficiency of an experiment could be measurably increased by introducing these factors associated with ovarian response into the design in a judicious manner. Such a design could eliminate much error variance in ovarian weight response to a gonadotrophin, thereby increasing the precision of results or allowing the use of smaller numbers for equivalent dependability. For example, in this colony if litter mates were paired between one group and another, the error variance would be reduced 58 percent in comparison to what it would be if body weight, time, heredity, etc., were completely ignored. That is to say, the dependability of a similar difference between means if litter mate pairing were used would be about 2.4 times as great when based on the same numbers or would require 58 percent fewer replications for the same accuracy.

In addition to the more immediate control of variation suggested by these results there is also the possibility of additional physical control of variation by eliminating that part due to heredity through an inbreeding program. It is not possible to predict the exact limits to which this reduction may go nor what changes may occur in the relative importance of the other factors as inbreeding proceeds, but theoretically about 28 percent of the variance ought to be eliminated by this procedure.

Another useful purpose served by these results is their prognostic value in the progeny-test selection project now under way on the descendants of the individuals reported here. The theoretical expectations as to the effectiveness of selection (combined with inbreeding in one group and on non-inbreds in another when both extremes are being selected for in each group) has been worked out from these same data and is now being subjected to a practical breeding check.

Another function that these results have is to narrow down the number of channels through which it would appear profitable to search experimentally for the physiological basis of differences between rats in their ovarian sensitivity. A comparable analysis should, of course, be made when the inbreeding has been completed, because it is on the divergent inbred lines that the most intense study will be undertaken.

Finally, it should be re-emphasized that these results apply specifically to a particular population, at a certain time, under the conditions specified. To what extent the results can be generalized is not known, but it seems unlikely that there would be any bias in these results, hence the general, if not the specific, picture should have wide application.

SUMMARY AND CONCLUSIONS

The relative importance of the genetic and nongenetic sources of variation in the weight response of the immature rat ovary to a standard dose of gonadotrophin in a colony of random bred animals maintained under relatively uniform conditions for a period of two and one-half years are approximately as follows:

Uncontrolled nongenetic factors associated with monthly fluctuations, such as changes in quality of feed, strength of hormone, and general management of the colony—17 percent.

Intra-month post-mortem body weight—13 percent.

Intra-month, intra-body-weight heredity—28 percent.

a. Additive scheme—25 percent.

b. Nonadditive allelic (dominance) deviations from the additive scheme—three percent.

c. Nonadditive, nonallelic (epistatic) deviations from the additive scheme—negligible.

Intra-month and intra-body-weight maternal factors—that is, those producing individual peculiarities of uterine environment and nursing ability of dam—six percent.

a. Permanent (those idiosyncrasies which carry over from one litter to another of the same dam) prenatal and postnatal—negligible.

b. Temporary (common to litter mates only) prenatal—negligible, postnatal—six percent.

Intra-month and intra-body-weight nongenetic, nonmaternal effects such as temperature, humidity, parasites, injection technique, etc., which operated

on all contemporary individuals alike and distinguished them from non-contemporaries—nine percent.

Environmental factors not even common to litter mates—namely, accidents of development which may be related to implantation, nursing, parasitic or bacterial infection, etc.—27 percent.

The application of these results to the design of experiments and selection procedures is discussed.

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CHROMOSOME VARIATION IN DROSOPHILA ATHABASCA

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INTRODUCTION

THE structural detail exhibited by the salivary gland chromosomes of the Dipterans has made possible extensive surveys of the chromosome variation in natural populations of Drosophila. Such studies (DOBZHANSKY and STURTEVANT 1938; DOBZHANSKY and SOCOLOV 1939; DOBZHANSKY 1944; MILLER 1939; CARSON and STALKER 1946) show that variability in the form of inverted sections of chromosomes is widespread; they have in addition revealed new problems with respect to the evolutionary significance of such variation.

Drosophila athabasca (STURTEVANT and DOBZHANSKY 1936a) seems well-suited for such an investigation. Its geographic distribution is one of the most extensive of the wild Drosophila species (fig. 1). It has been found in northwestern United States, southwestern Canada, southern Alaska, and in the eastern part of the continent, from Quebec to North Carolina. Complete data on its distribution in the middle section of the continent are lacking; it has been found, however, in Minnesota and in South Dakota. It seems quite likely that the range of *D. athabasca* is actually continuous across the continent.

All three species hybrids known in the *affinis* subgroup involve *D. athabasca*. It has been shown to produce viable offspring when mated with *D. azteca* (STURTEVANT and DOBZHANSKY 1936a), with *D. affinis*, and with *D. algonquin* (MILLER 1939 and unpublished). These facts, in combination with the data on the geographical distribution of all the members of this group, suggest that *D. athabasca* is the link by which some of the others are related, particularly the eastern (*D. algonquin*, *affinis*, *narragansett*, *seminole*) as opposed to the western (*D. azteca*, *dobzhanskii*) species.

The eastern form of *D. athabasca* had originally been separated from the western form as a subspecies, *mahican*, on the basis of a somewhat paler coloration (STURTEVANT and DOBZHANSKY 1936a). During the course of the investigations to be described, no evidence has been found that any distinction need be made between the two forms, and the subspecific designation has been disregarded.

MATERIAL

Strains of *D. athabasca*, derived from single fertilized females captured at various localities, have been established in the laboratory. Those strains analyzed are represented in figure 1 by closed circles at the locality from which they were taken. Table 1 lists localities of the strains analyzed, with the designation applied to those strains.

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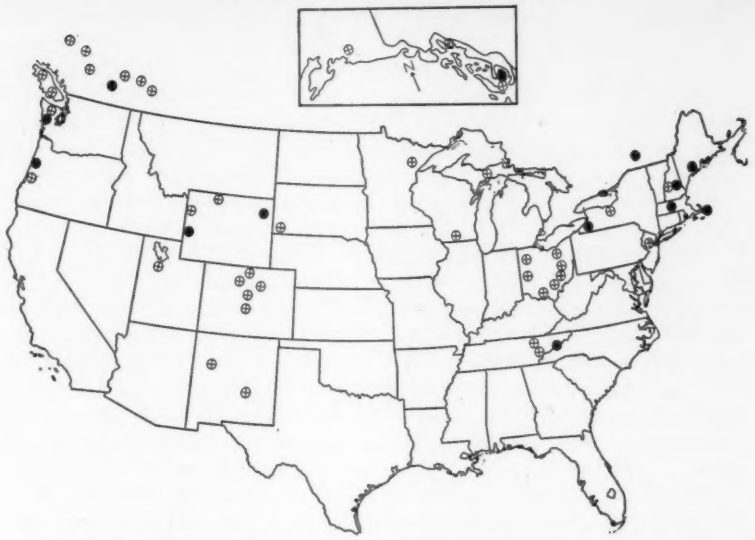


FIGURE 1.—Known distribution of *D. athabasca*. Black circles indicate localities from which strains have been established and analyzed. The insert is a view of southern Alaska.

METHODS

The analysis of the various strains of *D. athabasca* has been accomplished by a study of the salivary gland chromosomes of the larva. The detail obvious in preparations of those chromosomes makes it possible to distinguish small dif-

TABLE 1

The localities and designations of the strains analyzed.

LOCALITY	NUMBER OF STRAINS	DESIGNATIONS
Gravina Island, Alaska	1	Alaska
Quinault, Washington	1	Washington
Sundance, Wyoming	1	Wyoming-1
Grey's River, Wyoming	2	Wyoming-2, -3
Perpetua, Oregon	2	Oregon-1, -2
Hope, British Columbia	4	British Columbia-1, -2, -3, -4
Montreal, Quebec	1	Quebec
Gray, Maine	1	Maine
Franconia Notch, New Hampshire	2	New Hampshire-1, -2
Amherst, Massachusetts	1	Massachusetts-1
Woods Hole, Massachusetts	1	Massachusetts-2
Chautauqua, New York	1	New York
Clingman's Dome, North Carolina	1	North Carolina

ferences in the structure of the two strands which form the complete chromosome. Since each of the two strands comes from each of the parents of an individual larva, differences in the structure of the two reveal themselves as abnormal pairing tendencies. The most common type of difference in chromosomes derived from wild strains is the inversion, in which a section of one chromosome has become inverted with respect to the same section of the original. Thus, if a chromosome is arbitrarily numbered along its length 12345678, section 23456 may become inverted to produce a chromosome of the constitution 16543278. A larva which is heterozygous for both sequences will show, in its salivary gland chromosomes, a loop, formed by the two different strands as they attempt to synapse completely (see fig. 2B).

If two inversions occur successively and if the regions involved overlap on the chromosome, then a final sequence of sections results which enables one to predict the constitution of the first inversion, although it may never have been seen (STURTEVANT and DOBZHANSKY 1936b). A chromosome of the constitution 154762389 differs from the standard sequence 123456789 by two such overlapping inversions; the simplest and most probable explanation of the changes that must have occurred to produce the final doubly inverted sequence is that an intermediate step, 154326789, differing from "standard" by a single inversion, was itself modified by an inversion of section 3267. This type of analysis can be extended to include a large number of different sequences of the same chromosome; the phylogenetic relationship of those sequences can then be graphically represented on a chart.

In practice, double inversion differences other than overlapping ones can yield more or less comparable phylogenetic information. Two included or two independent inversions whose breakpoints are such that the probability that crossing over will either combine or separate them is negligible can be adapted to a phylogenetic series. In these cases it is not possible to say which of the inversions must have occurred first, but it seems clear that one of them did and that this ambiguous hypothetical sequence represents an intermediate step in the production of the double inversion.

Furthermore, two independent inversions which theoretically might undergo crossing over to produce various combinations of sequences may be separated geographically so that these combinations are never found. In this case the phylogeny of such inverted sequences is reasonably clear: the "standard" sequence is interposed between the inverted sequences. The argument of geographical isolation applies equally well to the hypothesis that one inversion may have arisen on a chromosome already inverted at a different region with crossing over subsequently separating the two. The assumption that the present known distribution of sequences corresponds to that at the time of origin of the inversions is one that must be examined critically for each case.

Since, in a study of wild populations, the "standard" sequence must be chosen arbitrarily, there is no reason to assume *a priori* that the direction of change is from the "standard" to the relatively inverted sequences and not vice versa. For this reason, the direction of change is generally represented by a double arrow (↔).

TECHNIQUE

The salivary gland chromosomes were stained, for the most part, with aceto-carmine; aceto-orcein was used during the latter part of the work. Favorable preparations of the salivary gland chromosomes of *D. athabasca* are fairly rare. In no case has it been possible to derive the essential characteristics of a given configuration from a single nucleus. Consequently, all the drawings presented here are composites taken from a number of different camera lucida drawings of a given configuration in various nuclei.

STRUCTURE OF THE CHROMOSOMES

The metaphase chromosome configuration of a *D. athabasca* male nerve ganglion consists of a V-shaped X chromosome, a J-shaped Y chromosome, three pairs of J-shaped autosomes, and a pair of microchromosomes (STURTEVANT and DOBZHANSKY 1936a). The salivary gland chromosomes of the male consist of two long pale strands (the X chromosome), three long and two short strands. One of the longer strands represents a complete autosome; two other autosomes are formed by a long and short strand each. The terminology used here for the chromosomes is that suggested by STURTEVANT and NOVITSKI (1941) for the *D. affinis* subgroup. The X chromosome is chromosome AD, the three autosomes are B, C and E (C being single-armed), and the microchromosome is F.

The drawings of the salivary gland chromosomes presented here are complete only insofar as they illustrate the gross structure of naturally occurring sequences. Chromosomes which show no variation have been illustrated by drawings of the basal and terminal regions for purposes of identification. The chromosomes which yielded phylogenetically useful information will be considered first.

SEQUENCES IN CHROMOSOME C

The sequence chosen as standard, for purposes of reference, was one found to be homozygous in the North Carolina strain and occurring also in the strains New Hampshire-1, -2, Maine, Massachusetts-1, -2, and New York. This sequence was arbitrarily lettered A. The sections into which it has been divided have as their endpoints the regions at which breakages of the chromosome by inversion are known to occur. This system of numbering facilitates the description of relatively inverted sequences in terms of sections. In the serial notation of sequences, hyphens are used to indicate the omission of consecutive sections and periods indicate the points at which breakage by inversion occurred.

A double inversion with respect to A has been found in Maine, both Massachusetts and both New Hampshire strains. It has the sequences:

1-7.11-23.10-8.24-28 (C)

This is either an included or an overlapping inversion (fig. 2A) with two of the breakpoints of the two individual inversions occurring in close proximity. No sequence has been found which might represent an intermediate step (B).

The strain of New Hampshire-1 yielded two simple inversions with respect to A (fig. 2B and C). The order of sections on the sequence is:

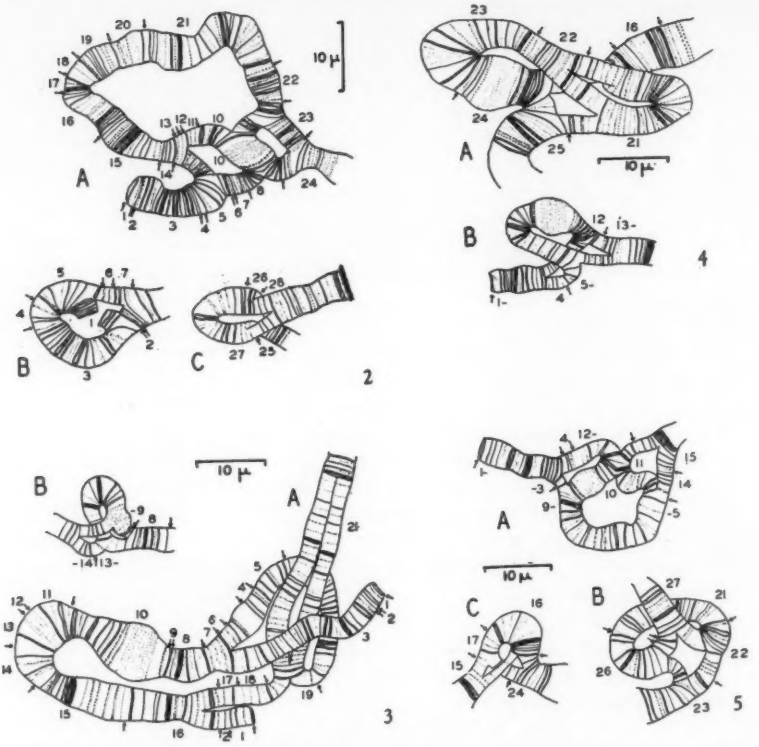


FIGURE 2 (upper left).—Inversions in chromosome C. A. A/C inversion. B. A/D inversion. C. A/E inversion.

FIGURE 3 (lower left).—Inversions in chromosome C. A. A/I inversion. B. A/J inversion.

FIGURE 4 (upper right).—Inversions in chromosome C. A. A/L inversion. B. A/M inversion.

FIGURE 5 (lower right).—Inversions in chromosome C. A. LM/O inversion. B. LM/P inversion.

1.6-2.7- (D)

and:

-25.27 26.28 (E)

Both were found together in combination with sequence C; since they do not overlap C and are sufficiently distant from it that crossing over may have combined them with C after their origin on another sequence, the exact phylogeny is not clear. The sequence actually observed was DCE (1.6-2.7.11-23.10-8.24 25.27 26.28).

In New Hampshire-2, a sequence of at least six breakpoints with respect to A has been found (fig. 3A). The sequence of sections is:

1 2.16-6.4 5.20.3.17-19.21-28 (I)

In order to derive this from A, which is the most closely related of the sequences, it is necessary to postulate four inversion steps. Two pairs of the breakpoints must occur in close proximity and have not been differentiated. The three hypothetical sequences between A and I are represented by the letters F, G, and H.

A small inversion found in the Massachusetts-2 strain (fig. 3B) differs from A by the following sequence:

1-8.13-9.14-28 (J)

The phylogenetic relationship is obvious, since it is a direct derivative of A.

In the Quebec strain a double inversion with respect to A is found in the proximal end of element C (fig. 4A). The sequence of sections of this relatively inverted chromosome is:

16 17.24-22.18-21.25-28 (L)

The intermediate step K to this double inversion has not been seen. All western forms show this sequence, or derivatives of it, in the basal part of chromosome C.

The western strains differ from the eastern ones by an additional distal inversion (fig. 4B). It has the sequence:

1-4.12-5.13- (M)

The LM combination of sequences (1-4.12-5.13-17.24-22.18-21.25-28) has been found in Alaska, Wyoming-1, British Columbia-1, -2, -4, Washington and Oregon-1. This combination represents the closest relationship of the western sequences to A. All other sequences found in the western range of *D. athabasca* are derivatives of LM.

A double inversion with respect to LM (fig. 5A) has been found in Washington, Wyoming-2, -3, and British Columbia-3. The order of sections on it is:

1-3.9-5 13 14 12.4.10 11.15-17 24-22 18-21 25-28 (O)

The intermediate step necessary to derive this overlapping inversion sequence from sequence LM is:

1-3.4 12 14 13 5-9.10 11 15-17 24-22 18-21 25-28 (N)

No sequence that might represent this intermediate has been observed. In order to test whether the standard A sequence was not, in fact, this hypothetical sequence, nuclei were obtained which were heterozygous for A and O. Instead of the single distal inversion expected if A were the hypothetical, the salivary gland chromosomes showed a triple distal inversion.

A simple inverted sequence with respect to LM (fig. 5B) found in British Columbia-1, -2, -3 and -4, Oregon -1 and -2 strains has the order:

-23.26 25 21 20 19 18 22.27- (P)

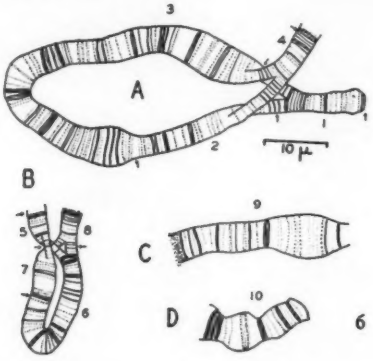
Combinations of sequences O and P can occur in the same chromosome, since the nearest breakpoints of the two independent inversions are sufficiently separated. The sequence OP (1-3 9-5 13 14 12 4 10 11 15-17 24 23 26 25 20 18

21 22 27 28) has been found in British Columbia-3, -4, and Oregon-1, and -2 strains.

SEQUENCES IN CHROMOSOME E

Chromosome E is composed of two arms, a long and a short one. The longer arm has been numbered from 1 to 8; no inversions have been found in the shorter arm, and it has consequently been numbered 9 at the basal region (fig. 6C) and 10 at the distal end (fig. 6D).

The sequence 1 2 3 4- (A) has been found in the following strains: North Carolina, New Hampshire-1, Maine, and Quebec. The relatively inverted sequence 1.3 2.4- (B) (Fig. 6A) has been found in New Hampshire-1, -2, New York, Maine, and Quebec in the eastern range. All the western strains studied are homozygous for this sequence.



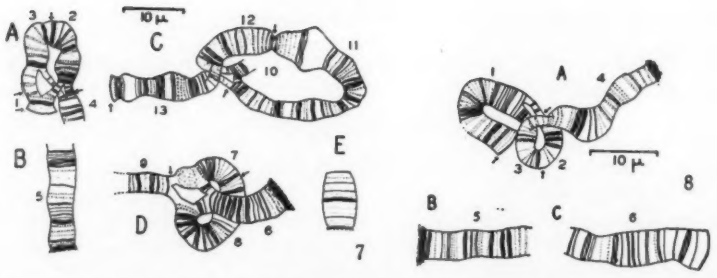


FIGURE 6 (above).—Sequences in chromosome E. A. A/B inversion. B. C/D inversion. C and D. Basal and distal ends, respectively, of the short arm.

FIGURE 7 (lower left).—Sequences in chromosome AD. A. A/B inversion in limb A. B. Basal region of limb A. C and D. Distal and proximal inversions, respectively, of the C/D complex in limb B. E. The microchromosome E.

FIGURE 8 (lower right).—Sequences in chromosome B. A. A/B inversion. B and C. Distal and proximal regions of the longer arm.

The sequence -5 6 7 8- (C) occurs in the western strains Alaska, Wyoming-1, -2, -3, Washington, British Columbia-1, -2, -3, -4, Oregon-1 and -2, and in the eastern strains North Carolina, New Hampshire-1, Maine, Massachusetts-1, -2, and Quebec.

The relatively inverted sequence -5.7 6.8- (D, fig. 6B) occurs in the western strains Washington, Wyoming-3, British Columbia-1, -3, -4, and Oregon-2; among the eastern strains it is found in New Hampshire-1, Maine, Quebec, and Massachusetts-2.

SEQUENCES IN CHROMOSOME AD

Elements A and D are combined in *D. athabasca*, as in *D. pseudoobscura* and other closely related forms, to form the X chromosome (STURTEVANT and NOVITSKI 1941). Limb A has been numbered from 1 to 5 proceeding proximally; Limb D has been numbered from 6 to 13 proceeding distally.

Only two arrangements have been found in the short arm A. Together they form a subterminal inversion (fig. 7A). The sequence 1.3 2.4 (B) is limited to the Alaska, Wyoming-1, -3, Oregon-1, and -2 strains. All other strains studied and also Wyoming-1 have the sequence 1 2 3 4 (A).

D, the longer limb, has as its most common sequence 6 7 8 9 10 11 12 13 (C). It has been found in all strains except New Hampshire-1, New York, and Massachusetts-2. These three strains, along with New Hampshire-2, showed a sequence differing from C by three independent inversions, the basal and distal of which are shown in fig. 7C and 7D. The sequence of these chromosomes is 6.8 7.9.....10.12 11.13 (D). Those inversions have not been found separately; it is possible that they form the "sex-ratio" complex, which was found genetically by STURTEVANT and DOBZHANSKY (1936a) in the Massachusetts-2 strain.

SEQUENCES IN CHROMOSOME B

Chromosome B has two unequal arms; the longer arm has as its most prevalent sequence one that is found in both eastern and western strains. The tips and base of this sequence are illustrated in figures 8B and C. Inversion configurations in this arm have been seen occasionally, but because of the rarity with which satisfactory preparations involving this arm are obtained, no attempt has been made to analyze those occasional variations.

Two sequences occur in the shorter arm (fig. 8A). The sequence 1 3 2 4 (A) occurs in all the strains examined except two; the relatively inverted sequences 1.2 3.4 (B) has been found in the Massachusetts-1 and -2 strains.

THE PHYLOGENY OF THE SEQUENCES IN CHROMOSOME C

As has been pointed out previously, the actual order in which the various sequences have originated is not immediately obvious from their relationships as sequences. It is pertinent to attempt to establish such an order so that the geographical distribution of the various sequences may be interpreted in terms of the history of the species itself. The probable base of the phylogenetic tree of sequences (fig. 9) has been arrived at simply on the basis of the geographical

distribution. The eastern and western forms of *D. athabasca* have probably been isolated from each other for a long period in their history, although not long enough nor completely enough for their divergence into separate species. Any sequence that they might have in common would seem to be the primitive sequence or a close relative of it. Actually they have none in common, but each of the two forms has as its most prevalent sequence two that are very closely related (*A* and *LM*). (The letters which represent known sequences are italicized; letters representing hypothetical sequences are not.) It seems likely that one of these two, or one of the intermediates between them (K, *L*, or M)

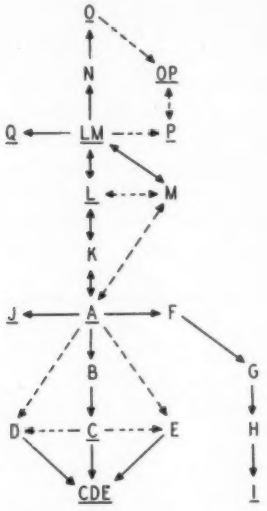


FIGURE 9.—The phylogenetic tree of sequences in chromosome C. Underlined sequences are known ones; the others are hypothetical. Dotted lines indicate ambiguous paths in the phylogeny.

represents the original type. All the arrows between these, consequently, are doubly pointed. Further derivatives of these are designated by arrows pointing away from this group.

Starting with the "standard" *A* sequence, *J* differs from it by a singly inverted section. The relationship is *A*→*J*. (Frequent references to figure 9 may be helpful during the following discussion.) Sequence *I* differs from *A* by four consecutive inversions; three hypothetical intermediates must come between *A* and *I* (*A*→F→G→H→*I*). Between *A* and the doubly inverted sequence *C* is the hypothetical B (*A*→B→*C*). The sequence *CDE* differs from *C* by two independent inversions, D and E. Since crossing over might have combined them with C after their origin on another sequence, as *A*, the exact phylogeny is ambiguous. Either may have originated from *A* or *C* (*A*→D or *C*→D and *A*→E or *C*→E), but all three, *C*, D, and E have been combined to produce *CDE*

(D→*CDE*←E). Ambiguous possibilities are included in figure 9 with interrupted arrows.

Sequence *L* differs from *A* by two inversions, the hypothetical intermediate K has not been seen (*A*→K→*L*). Sequence M does not occur alone but occurs only in combination with *L* as *LM*. Since crossing over may have combined M with *L* after its origin on another sequence such as *A*, the origin of *M* is ambiguous and can be represented thus: *L*→M←*A*. *L* and M, however, together produce the widely distributed *LM* (*L*→*LM*←M). The derivation of the singly inverted sequence *Q* can be simply represented as *LM*→*Q*. *O*, a double inversion with respect to *LM*, has a hypothetical intermediate N (*LM*→N→*O*). The origin of *P* is ambiguous: It occurs both with and without sequence *O*, and since there is ample opportunity for crossing over either to combine or to separate them, *P* may have arisen on the *LM* sequence or it may have arisen on the *O* sequence to give *PO*, later separating from *O*. The various paths that these sequences may have taken are illustrated in figure 9.

THE GEOGRAPHICAL DISTRIBUTION OF THE VARIOUS SEQUENCES

The number of strains analyzed has not been great enough to establish with a high degree of certainty the absence of any given sequence in any particular area in which *D. athabasca* is found. A comparison of the eastern and western strains, however, shows significant trends which are not likely to be altered by a study of additional strains.

All the chromosomes, except C, have at least one sequence common to both eastern and western strains. Chromosome E has two such sequences, BC and BD. It seems not unlikely that these widespread sequences are older than those which are found in only a limited region and are actually the primitive sequences in existence at the time of the original migration of the species.

In the case of chromosome C, however, no sequence is common to both East and West. Furthermore, the variations found in each of the two ranges are closely related to others of the same range, but are more distantly related to those occurring on the other side of the continent (fig. 10). This leads to the conclusion that the variations have arisen after *D. athabasca* had spread to its present distribution. Since strains from the extremes of the range (as the Alaska and North Carolina strains) show no evidence of incipient speciation, as sexual isolation or infertility of offspring when mated together, and show only a slight undependable morphological difference, it appears also that rate of inversion formation in this species is rapid compared to speciation. While it is difficult, if not impossible, to refer the migration of the species and origin of the inverted sequences to specific geological periods, it can be broadly stated that the evidence favors a theory of recent origin of inversions in *D. athabasca*. (See EPLING (1944), MAYR (1945), STEBBINS (1945), and SIMPSON (1945) for a consideration of the ages of the gene arrangements in *D. pseudoobscura*.)

A THEORY OF THE ORIGIN OF CERTAIN INVERSIONS

Chromosome C of *D. athabasca* shows a disproportionately high variability as compared with the other chromosomes. It is homologous to the chromosome

which has the greatest variability in *D. pseudoobscura* (DOBZHANSKY and STURTEVANT 1938; STURTEVANT and NOVITSKI 1941). In *D. azteca*, the chromosomes homologous to C and E show a relatively high variability compared to the others (DOBZHANSKY and SOCOLOV 1939), whereas in *D. algonquin*, all the chromosomes have a uniformly low variability (MILLER 1939). These numerical relationships are presented in table 2. It is interesting to note that *D. affinis*,

FIGURE 10.—The geographical distribution of the various sequences of chromosome C.

for which no cytological data are available, agrees with *D. athabasca* and *D. pseudoobscura* in the distribution of inversions among the chromosomes. STURTEVANT (1940) says of the X chromosome, "Two different sequences occur in each arm"; of chromosome E, "No variations in linkage have been found, indicating that the sequence . . . is the usual one throughout the range of the species"; of chromosome B, " . . . shows values (of crossing over) that have been obtained repeatedly from crosses to a wide variety of strains"; but of chromosome C, "The possible combinations of three . . . characters . . . have been studied rather extensively but no double recessive has yet been obtained. Either all three loci are close together, or the chromosomes concerned have different sequences."

The reason certain chromosomes are more variable than others has not been satisfactorily explained. One possible answer is that the more variable chromosomes differ from the others in possessing a higher inherent "breakability." HELFER (1941), in a study of the aberrations produced by X-irradiation of *D. pseudoobscura*, found no differences in "breakability" of the various chromosomes.

For a clue to the mechanism by which inversions arise in a natural popula-

tion, it is pertinent to examine in detail the distribution of breakpoints along the length of the chromosome. Figure 11A shows the breakpoints of the known inversions in chromosome C of *D. athabasca*. The euchromatic length of that salivary gland chromosome is depicted schematically by a line, with basal heterochromatin at the right. If it is divided into four equal parts, ten breakpoints occur in the most distal quarter, nine in the second, three in the third, and five in the basal quarter (none occur, however, in heterochromatin). The distal clustering of breakpoints is obvious.

TABLE 2

A comparison of the numbers of different sequences found in the chromosomes of D. athabasca and the homologous chromosomes of closely related species.

SPECIES	ELEMENTS				
	A	B	C	D	E
D. athabasca	2	4	17	2	2
D. algonquin	3	3	4	1	4
D. azteca	2	1	6	3	6
D. pseudoobscura	2	2	22	3	6

For the purpose of this analysis, the third chromosome (element C) of *D. pseudoobscura* serves better than that element of *D. athabasca* because of the extent of the phylogenetic tree and the absence of large numbers of hypotheticals. Since these breakpoints have been related to "standard" sequence, and inversions differing from standard by more than one step originate on sequences which have sections "out of order," the position of the breakpoints

FIGURE 11.—A diagrammatic representation of the euchromatic length of chromosome C in *D. athabasca* (A) and *D. pseudoobscura* (B). The basal heterochromatin is represented by a tuft at the right end. The vertical lines represent the positions of inversion breakpoints.

may be recalculated with respect to their relative position at the time of the formation of the inversion (DOBZHANSKY 1944). Figure 11B shows the schematic representation of the positions at which the chromosome "broke" by inversion. Fourteen breakpoints fall in the distal quarter, 11 in the second, five in the third, and two in the basal quarter. Here, as in *D. athabasca*, none occurs in heterochromatin. The deviation from a random distribution is quite significant.

Elements C of *D. pseudoobscura* and *D. athabasca* show the same distal clustering; since each must differ from the other by many inversions, with a consequent relative scrambling of sections, the distal clustering can hardly be the result of a higher inherent "breakability" of specific regions but is more likely a property assumed by sections which happen to fall in the distal region.

Furthermore, if an inversion occurs on a sequence which itself is inverted with respect to a more primitive sequence, its breakpoints tend to bear a definite relation to those of the parent inverted sequence. Thus, in chromosome C of *D. athabasca*, sequences C, and L differ from A by double inversions in which the two most nearly proximal breakpoints of the individual inversions occur so close together that they could not be distinguished in the preparations studied. It might be considered that these configurations were in fact transpositions of sections were it not for the fact that the supposedly transposed section in both cases occurs in reversed order. Furthermore, a transposition of a section would require the simultaneous occurrence of three breaks; a double overlapping inversion requires two sets of two breaks each, with each set occurring at a different time. The probability of the former in nature seems much more unlikely than the latter.

In the *D. pseudoobscura* phylogenetic series of sequences in the third chromosome, there are two branches with multiple derivatives each, involving no hypotheticals; A. Santa Cruz→Tree Line→Olympic (1), Oaxaca (2), and Estes Park (3), Hidalgo (4); B. Standard→Klamath→Sequoia II (1), Wawona (2), and Cowichan (3). If the salivary gland chromosome is represented by a line (fig. 12A and B) with the basal heterochromatin at the extreme left, the arrows above the line indicate the positions of the breakpoints which yielded the parent sequences Santa Cruz (A) and Klamath (B). The pairs of connected arrows below the lines representing the chromosomes indicate the points at which the already inverted sequences must have been broken to produce the derived sequences. The numbers after each sequence so indicated correspond to the numbers given to the sequences above. The distribution is obviously not random with respect to the inversion in the parental sequences.

The relation of the position of the breakpoints which produced the derived sequences to those breakpoints which produced their predecessors can be stated as follows: There is a distal shift in position of the breakpoints of the derivative inversions with respect to those of the original, so that the proximal breaks of the derivatives lie between the two breaks of the parental inversion, while the distal breaks occur beyond the distal break of the parental inversion. Of the 14 breakpoints considered in diagrams A and B of figure 12, only one violates this rule and it by about .04 of the euchromatic length.

The most obvious situation in which the breakpoints of an inversion are differentiated from other sections is that found during prophase of a cell heterozygous for an inversion. The tendency for complete synapsis of the two different sequences results in an asynapsis of the regions at which the inverted segment begins and ends.

Figure 12C is a simplified schematic representation of such pairing in a het-

erozygote for sequences ABCDEFGHIJKLMN and AB.KJIHGFEDC.LMN. It is depicted in the two-strand state, which condition will be assumed throughout this discussion for the sake of simplicity. This configuration leads to a juxtaposition of two unsynapsed sections that would ordinarily occur at random within the cell; these sections are those containing the breakpoints of the inversion (fig. 12D). Breaks in this region of the two single strands, followed by a refusion of the pairs of broken ends on the same sides (fig. 12E) would lead to the production of another inversion on either sequence depending upon

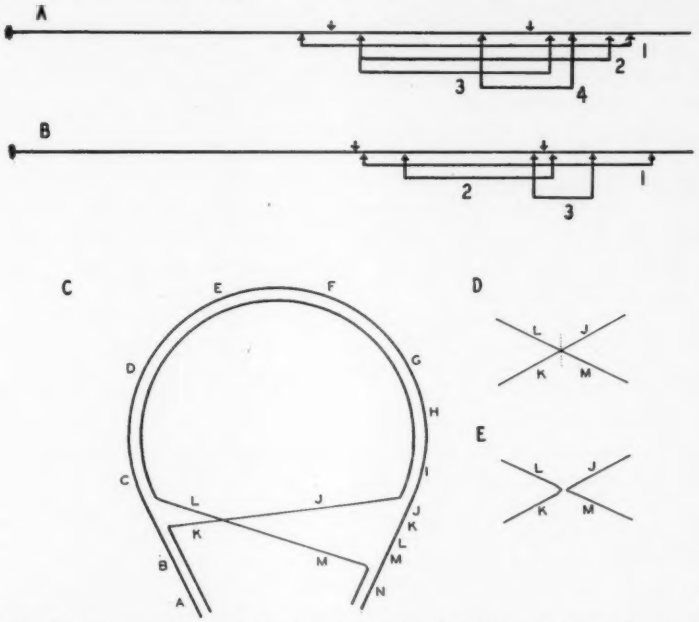


FIGURE 12.—A and B. The relation of the breakpoints of double inversions to those of their immediate predecessors. C, D and E. A suggested theory for the origin of certain inversions which show a non-random distribution of breakpoints. For complete explanation, see text.

which strand crossed itself. If it occurred on the uninverted sequence, a "mimic" inversion would be produced; if it occurred in the already inverted sequence, a derivative of this inversion would be formed. In the example given in figure 12, the new sequence formed is ABK.LCDEFGHIJ.MN, an inversion that overlaps with a distal shift the first inversion. The predominance of distal over proximal shifts in the inverted sequences found in nature suggests that synapsis tends to be more nearly complete proximally than distally—namely, that the single strands available for breakage and reunion are more apt to represent distal sections than proximal ones.

The question of the cause of breakage of the single strands has two possible

answers. The first is that breaks originating in the same manner as those that produce all natural inversions are here more likely to produce an inversion than they would ordinarily because of the proximity of the strands. Secondly, the tendency of the inverted sequences to pair may impose a strain upon the unsynapsed sections of the chromosomes. This strain may be relieved by a sort of "illegitimate crossing over" at a point of contact of the two strands (fig. 12C and D), or both strands may be broken to relieve the tension, with a subsequent uniting of broken ends on the same side.

Many of the sequence combinations found in nature do not have single inversion differences. Whether or not more complex configurations may be effective in the above scheme should depend, for each case, upon the location of the unpaired strands with respect to each other. The greater lack of pairing in multiple inversion heterozygotes might contribute to the availability of single strands available for breakage, but, on the other hand, this same lack of pairing should decrease the extent to which nonhomologous sections are opposed. Thus in the extreme case of a complete absence of pairing (which is quite common where two chromosomes differ by many inversions) the suggested mechanism for the origin of some of the inversions in *D. athabasca* and *D. pseudoobscura* would be inapplicable. An examination of multiple inversion configurations reveals that of the greater number of sets of strands thrown into apposition by close pairing of homologous sections, more than a third involve sections of different chromosomes, a breakage and reuniting of which would inevitably result in duplication and deficient or dicentric and acentric chromosomes. Therefore the elaboration of the scheme to include differences greater than a single inversion does not seem practicable. It might be suggested, however, that because of the relatively smaller length of paired sections within multiple inversion heterozygotes compared to simple inversion heterozygotes, any new inversions formed in the suggested manner would be comparatively small ones.

Additional evidence for the support of the suggested mechanism of breaks may be obtained by considering the case of *D. miranda*, a very close relative of *D. pseudoobscura*. In this species, chromosome X_2 represents a combination of a third chromosome (again, element C) and a Y chromosome, with successive inversions intercalating the Y heterochromatin sporadically along the euchromatic length of the autosome (MACKNIGHT 1939). Each successive inversion must involve one heterochromatic and one euchromatic break to produce these intercalations. This is in agreement with the trend of successive inversions in the obscura group to show a coincidence of breakpoints.

In seems likely that the suggested method of breakage is not the type that ordinarily produces inversions, for then it would be expected that all species which show variability in sequence (and all those studied do) would tend equally to have high variability. The difference between *D. pseudoobscura*, *D. athabasca*, *D. affinis*, *D. azteca*, and other species as *D. melanogaster*, *D. robusta*, *D. virilis*, etc., indicates that this is not so. Also, different chromosomes within the same species show unequal variability. The proposed theory explains the nonrandom distribution of breaks only.

SOME ASPECTS OF INVERSION POPULATION MECHANICS

Inspection of the phylogenetic charts of *D. athabasca* and *D. pseudoobscura* shows that the most common sequences (at the present time) are not also those with the greatest number of derivatives, but that the development of inversion differences has tended to proceed serially along a single path. This absence of bunching of derivatives near the base of the phylogenetic tree may be explained in part by considering separately the frequency of origin of derivative inversions and the chance of their incorporation into a population.

If a new arrangement is produced by a single reversal of the order of part of a chromosome irrespective of the nature of the homolog of that chromosome at the time of formation of the inversion, the frequency of origin of derivatives should be directly proportional to the frequency (p) of the original arrangement. If, on the other hand, inversions arise in cells heterozygous for previously existing inversions, then the frequency of origin should be proportional to pq, where p and q represent the frequencies of arrangements with a single inversion difference. Thus a population homozygous for one sequence would have a maximum potentiality in the first case and a minimum in the latter. The assumption of heterozygote-origin therefore may obviate the logical necessity of a one-to-one relationship between frequency (or, more precisely, age in time times frequency per unit of time) and number of derivatives.

Secondly, the double inversion differences considered here are of the overlapping type, which may give rise to duplication-deficiency gametes if a single crossover occurs within the region inverted in both arrangements. Since two chromosomes, one of each type, are lost in inviable zygotes by such crossing over, the less frequent one is at a selective disadvantage, in percentage terms, with respect to the more frequent one, other things being equal. Since the genic constitution of the derivative is that of its predecessor, there can be no immediate advantage due to heterosis. The selective disadvantage may be defined as $k_1pq_1+k_2pq_2+k_3pq_3 \ldots$ whose p is the frequency of the derivative, q_1, q_2, q_3, etc., represent the frequencies of arrangements which have an overlapping relationship with p and k_1, k_2, k_3, etc., represent the proportion of zygotes lost by single crossovers in the respective heterozygotes. Assuming, for simplicity, equality of the constants k_1, k_2, k_3, etc., it is clear that the selective disadvantage of an arrangement is proportional to the number and frequency of those other sequences with which it has an overlapping relationship. Since, at the base of a phylogeny, there are more sequences which have a double overlapping relationship with respect to a new derivative (more q terms) than at the extremities and the frequency of those sequences is usually higher (larger q values), the net result should be a more rapid elimination of derivatives near the base of the phylogenetic tree than of those at the extremities.

SUMMARY

A salivary gland analysis shows that variation in gene arrangement occurs in all the chromosomes of *D. athabasca*.

One chromosome, homologous to the highly variable third chromosome of *D. pseudoobscura*, has a disproportionately large number of different gene ar-

rangements. The phylogenetic derivation of these arrangements is presented.

A comparison of the arrangements in the eastern and western strains of *D. athabasca* shows that the sequences of the most variable chromosome are probably of recent origin.

An analysis of the position of the breakpoints in *D. athabasca* and *D. pseudoobscura* suggests that new inversions of the variable chromosomes are formed during the prophase of a cell which is heterozygous for an inversion.

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The writer wishes to express his appreciation for helpful advice, criticism and contributions of material to PROF. A. H. STURTEVANT and DR. D D. MILLER of this laboratory, to PROF. TH. DOBZHANSKY of COLUMBIA UNIVERSITY, PROF, J. T. PATTERSON of the UNIVERSITY OF TEXAS, PROF. W. P. SPENCER of the COLLEGE OF WOOSTER, and DR. P. T. IVES of AMHERST COLLEGE.

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MINIATURE SEED—A STUDY IN THE DEVELOPMENT OF A DEFECTIVE CARYOPSIS IN MAIZE

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INTRODUCTION

THE normal product of fertilization in maize is a mature dormant seed with a well developed embryo and starchy endosperm. All the many variations from this norm have been in a minus direction with regard to embryo or endosperm development and must be regarded as defective types. These defective types may be divided superficially into two groups on the basis of comparison of their growth to that of plants of similar genetic constitution but carrying the normal allele of the gene in question. The first group would comprise those defectives in which the homozygous defective plants are approximately equal in vigor, size, and maturity to the normal, although they may be somewhat smaller in early growth stages. The second group would include all the lethals, semi-lethals, and stocks in which the factor in question finds expression throughout the sporophytic generation and makes the plants shorter, lighter in color, more slender, and generally less vigorous.

Defectives of the first class are waxy, sugary, shrunken, and miniature germ. All these genes approach normal very closely in seed development as judged by weight. Waxy and sugary are products of upsets in carbohydrate metabolism—waxy corn forming exclusively amylopectin instead of a mixture of both amylose and amylopectin as in ordinary maize (SPRAGUE, BRIMHALL and HIXON 1943), while sugary cannot synthesize glucose into maize starch (EAST and HAYES 1911). WENTZ (1924) reported that miniature germ with a very small embryo has low germination and is weak in early growth stages but recovers to make a normal, mature plant.

The second group of defectives includes many genes, most of which form seeds with only two to 30 percent of normal development. Many workers ascribed such deficiency to faulty pollination or arrested development due to competition and dominance. However, JONES (1920) showed a heritable basis for some of these defects—stocks where development of the embryo and endosperm stopped completely shortly after fertilization. Since that time many deficiencies have been shown to be heritable. MANGELSDORF (1926) made a comprehensive study of 14 defective seed characters. These stocks ranged from those in which development was about 57 percent of that in normal seeds, and homozygous plants although stunted could be grown (*de2*), to one where development is about three percent of normal and viability is nil (*de14*). MANGELSDORF found a good deal of correlation between endosperm and embryo development in all his stocks. There was no satisfactory development of one without the other.

Other investigators have not always confirmed this in other defectives.

EYSTER (1931) found in the reduced endosperm stocks that the embryo showed almost normal development while the endosperm was nearly lacking. DEMEREC (1923) has reported a germless condition where kernels possessing a well-developed endosperm lacked an embryo. His genetic studies have shown that at least four genes are concerned in this situation.

Some of the defectives in this group approach normal closely in weight. Disintegrating endosperm (ROBERTS 1938) is about 98 percent by weight of the normal. This character shows a complete breakdown of carbohydrate metabolism when in the late milk stage. By an autolytic process a cavity is

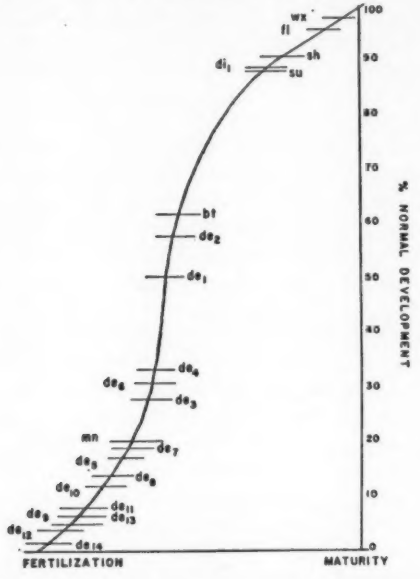


FIGURE 1.—Percentage of normal development of defective seed characters.

formed in the middle of the endosperm. Associated with this self-digestion is a swollen and distorted embryo.

MINIATURE SEED

The defective in this study, designated as miniature seed (*mn*), on the basis of growth, belongs to the first group, since plants from the miniature seeds are equal in final growth to those from normal seeds. However, it is the only member of this group which falls below 85 percent of the weight of the normal seeds of similar constitution. Miniature seed is only 20 percent by weight of normal seeds and, as may be seen by reference to MANGELSDORF's table of defectives (fig. 1), it occupies a position on the scale of normal development well below many of the defectives whose viability is very poor or almost nil.

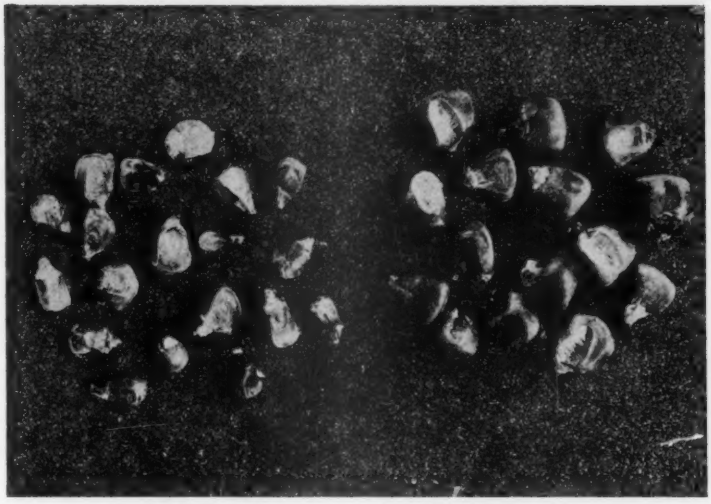


FIGURE 2.—Photograph of miniature seeds (left) and normal seeds (right).

Miniature seed which is 20 percent by weight of normal seeds represents an end product in which both embryo and endosperm are much reduced yet are present in about the same ratio as in normal seeds (table 1). The pericarp stimulated by fertilization outstrips the new tissue so that an easily-broken, papery covering characterizes the seed (fig. 2). The starch in the miniature is identical with that in normal seeds. A normal aleurone layer is developed, and corneous starch is present as in normal seeds. Genetically, miniature seed is a single factor difference, recessive to normal but epistatic to brittle and sugary.

TABLE 1

	65 NORMAL SEEDS		65 MINIATURE SEEDS	
	WEIGHT IN GRAMS	% TOTAL WEIGHT	WEIGHT IN GRAMS	% TOTAL WEIGHT
Embryo	3.3	12.34	0.75	13.74
Endosperm	23.6	87.66	4.71	86.26
Total	26.9		5.46	

In spite of small size, miniature seed germinates well. In one test seeds were taken from an ear segregating for normal, miniature, and brittle kernels. The normal seeds germinated 100 percent, miniature 98 percent, and brittle only 68 percent. Due probably to lack of food, homozygous miniature plants start more slowly than either heterozygous or normal plants. This difference per-

sists until flowering time when the miniature begins to approach normal in height. When mature, there is no significant difference in height, or in diameter of stalk (fig. 3). There is a significant difference in weight of ear and total plant weight due to the much smaller weight of the miniature seeds on the ears of recessive plants.

Further, it is possible to show that the tubes of pollen grains carrying the miniature allele are competitively equal to those carrying the normal allele.

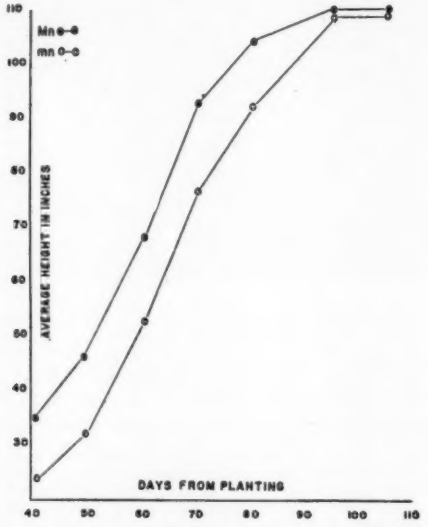
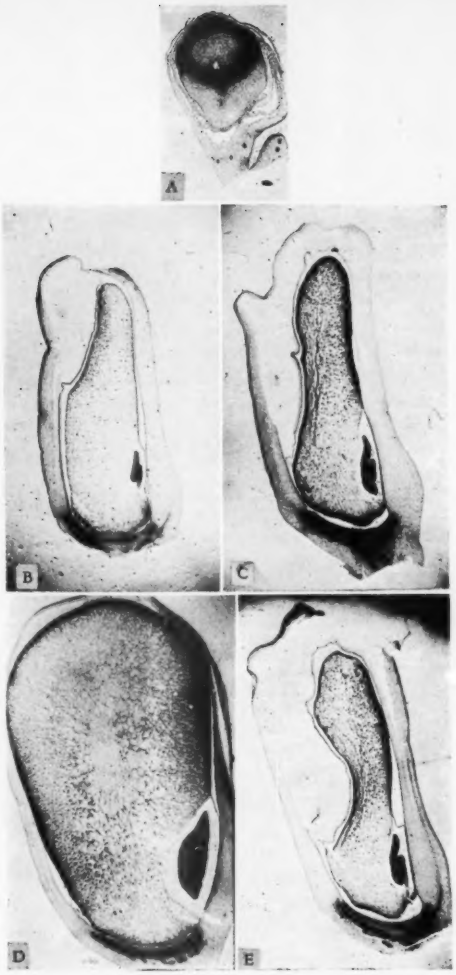


FIGURE 3.—Height of plants from normal and from miniature seeds plotted against days from planting.

Nineteen ears on plants of *mn*/+ genetic constitution were self-pollinated the day after the silks had been cut back almost to the end of the cob. After harvest the ears were divided into equal halves by the aid of a ruler, and the miniature and normal seeds in both the upper and lower halves counted. The total number of all the seeds was 8,929, of which 2,296 or 25.65 percent were miniature. The total number of seeds in the lower halves was 4,419, of which miniature numbered 1,148 or 25.97 percent. In the upper halves the total number of seeds was 4,510, with miniature numbering 1,148 or 25.46 percent.

Had this gene miniature extended its effects to the pollen tube, a significant difference between the ratios of recessive (*mn*) to dominant (++ and +*mn*) in the upper and lower halves of the ears would be expected. The chi squares for the upper and lower halves respectively are .496 and 2.25, showing that the small discrepancies obtained might easily be due to chance alone.



EXPLANATION OF PLATE I

All magnifications 12×.

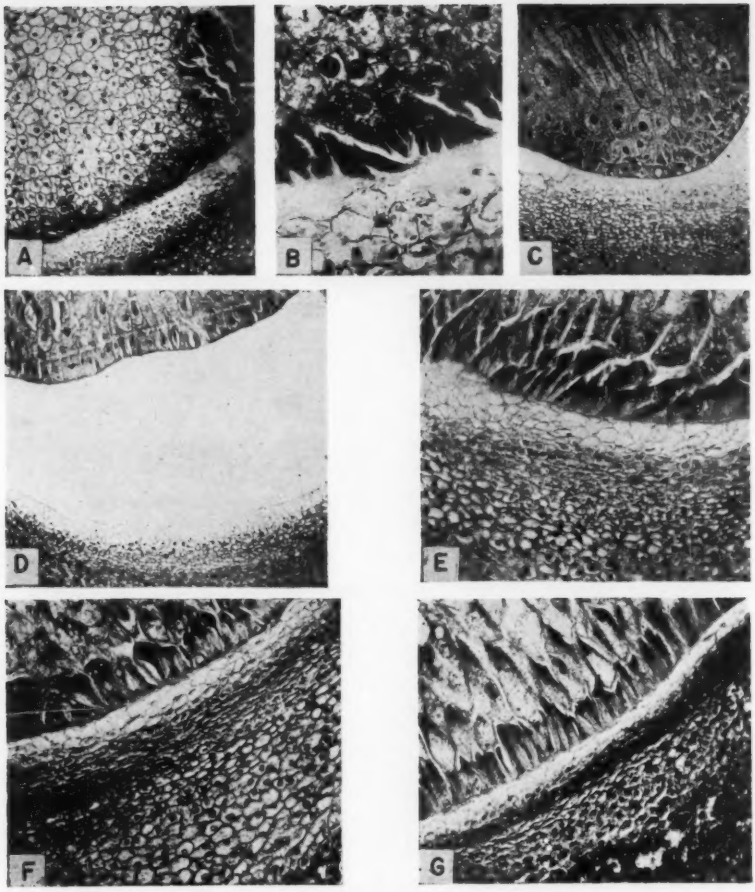
FIGURE A.—Cross section of a seed of corn two days after pollination, showing ovary wall, nucellus, and embryo sac.

FIGURE B.—Cross section of a normal seed 12 days after pollination.

FIGURE C.—Cross section of a miniature seed 12 days after pollination.

FIGURE D.—Cross section of a normal seed 19 days after pollination.

FIGURE E.—Cross section of miniature seed 19 days after pollination.



EXPLANATION OF PLATE II

In all the figures the endosperm of the seed is at the top, and the maternal tissue is at the bottom of the photograph.

FIGURE A.—Cross section of a miniature seed nine days after pollination. The endosperm is well developed, and its differentiated absorptive basal cells are in contact with the chalazal layer which serves as a bridge for nutrient supply between the conductive elements in the maternal tissue and the endosperm. However, the photograph shows that the chalazal cells are beginning to break down. 99×.

FIGURE B.—Same section as in figure A, but at a higher magnification to show more clearly the disintegration of the chalazal cells. 490×.

FIGURE C.—Cross section of a miniature seed 14 days after pollination showing the slight contact between the endosperm and the few remaining chalazal cells. 99×.

FIGURE D.—Cross section of a miniature seed 17 days after pollination showing the complete gap between the endosperm and the maternal tissue. 99×.

FIGURE E.—Cross section of a normal seed 12 days after pollination, showing a complete connection between the endosperm and the maternal tissue by the intact chalazal tissue. 200×.

FIGURE F.—Cross section of a normal seed 14 days after pollination, showing the intact layer of chalazal cells between the endosperm and the maternal tissue. 200×.

FIGURE G.—Cross section of a normal seed 19 days after pollination, showing that the chalazal layer is still completely intact. 200×.

HISTOLOGY

Materials and Methods

Kernels were collected from ears segregating *Mn* and *mn* seeds in a 1:1 ratio at intervals of two, five, nine, 12, 14, 17, and 19 days following pollinations; also from homozygous *mn* ears seven and nine days after selfing. The material was fixed in CRAF and dehydrated in an ethyl-butyl alcohol series according to the method of RANDOLPH (1935), embedded in paraffin, sectioned at 12 micra, and stained with Delafield's haematoxylin. Photographs of entire kernels were taken with the aid of a Bausch and Lomb dissecting microscope; close-up views of internal structures were made with a Bausch and Lomb compound microscope.

General Development of the Normal Seed

The mature ovule, which almost completely fills the ovarian cavity, consists of the many-celled nucellus, with its inner and outer integuments, and the embryo-sac embedded in and occupying a relatively small portion of the nucellar tissue. At the time of pollination this sac contains two synergid nuclei near the micropylar end, the egg cell, the two polar nuclei not yet fused, and a number of antipodal cells at the end opposite the micropyle. From 23–28 hours after pollination the pollen tube reaches the micropylar end of the ovule, enters the embryo sac, and discharges the two sperm nuclei, one of which unites with the egg cell to form the diploid zygote; the other with the two polar nuclei to form the triploid primary endosperm nucleus. Within two to four hours after fusion, this nucleus starts dividing so that by the time of the first division of the zygote, 10–12 hours following fertilization, there are usually four or eight free endosperm nuclei in the embryo sac. The endosperm expands rapidly, digesting away the surrounding nucellar tissue until only a thin layer of cells remains at the periphery. Both embryo and endosperm continue to grow and differentiate until the seed is mature, about 45 days after pollination (WEATHERWAX 1923; MILLER 1920; RANDOLPH 1936). See Plate I, fig. A, B, D.

Development of Miniature Seed

Early stages in the development of *mn* kernels appear perfectly normal. However, microscopic study of sections from seed nine days after pollination show that the cells of the chalazal pocket of the ovule are becoming empty and that their cell walls are breaking down. This layer of chalazal tissue, in the normally growing caryopsis, serves as a bridge for the passage of nutrients from the vascular bundles in the ovary wall to the well differentiated, densely-cytoplasmic, absorptive cells at the basal region of the endosperm. Sections of later stages of *mn* show a progressive disintegration of the chalazal cells. At 14 days after pollination there is only a very slight connection between the basal endosperm cells and the chalazal pocket, and at 17 and 19 days, the gap is complete. An arrest in growth in endosperm and embryo accompanies this

break-down of chalazal tissue. Sections from *Mn* kernels at comparable stages show that the chalazal tissue completely bridges the area between the vascular elements in the ovary and the absorptive cells at the base of the endosperm, and that the endosperm and embryo continue to enlarge (Plate I, fig. B–E; Plate II, all figures).

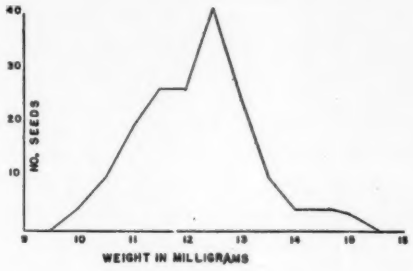
DISCUSSION

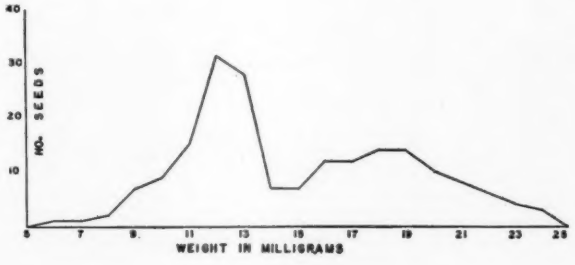
Through the recent studies of COOPER and BRINK (1940) and BRINK and COOPER (1941) on seed failure both in interspecific crosses in tobacco and in self-fertilization in the normally cross-pollinated alfalfa, more and more has it been realized that normal development of the endosperm is extremely important for the normal development of the embryo and the entire seed. Sections from seeds in the completely incompatible cross *Nicotiana rustica*×*Nicotiana glutinosa* and from the highly sterile cross *Nicotiana rustica*×*Nicotiana tabacum* reveal a retarded growth of the endosperm; failure of formation of conducting tissue between the vascular bundle of the integument and the chalazal pocket; prevention of contact between the basal endosperm cells and the chalazal pocket by the hyperplastic activity of the nucellus; and death of the embryo following disintegration of the endosperm. A similar situation occurs in self-fertilized alfalfa. Endosperm breakdown accompanied by proliferation of the surrounding maternal tissue leads to the collapse of 34.4 percent of the fertile ovules; after cross pollination only 7.1 percent of the fertile ovules abort. These investigators believe that the endosperm must be so constituted genetically that it can develop fast enough to keep an upper hand over the surrounding maternal tissue; otherwise, this tissue utilizes an abnormal proportion of the available food supply, thereby starving the endosperm and its nursling, the embryo, to death.

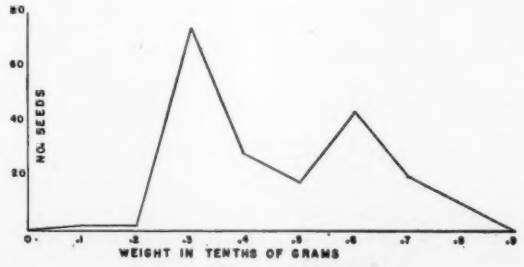
In tetraploid-diploid, and in incompatible species crosses in Datura, the situation is somewhat different in that sometimes it is the proembryo and sometimes the endosperm which collapses first, as maternal tissue proliferates (SANSOME, SATINA, and BLAKESLEE 1942).

In the present investigation of miniature seed, histological evidence shows that development of the young caryopsis is normal till the ninth day after pollination. Apparently this regular initial growth of both endosperm and embryo prevents excessive proliferation of maternal tissues, as occurs in sterile tobacco and Datura crosses. However, at the ninth day, the chalazal layer of cells connecting the vascular elements of the ovary and the absorptive cells of the endosperm begins to disintegrate. This breakdown of chalazal tissue continues until by the seventeenth day the gap between the conducting cells of the maternal tissue and the endosperm is complete. In normal seeds the chalazal bridge remains intact. Therefore, the small size of the mature miniature seed is presumably caused by partial starvation of the endosperm and embryo. Nevertheless, before the chalazal gap is complete, either enough nutrients have already been absorbed by the endosperm to allow it and the embryo to mature with little further growth, after the 14th day, or else the endosperm can still obtain a limited amount of food from the side walls of the ovary (Plate I, fig. B–G).

The situation suggests that the normal endosperm tissue produces some substance which either promotes longevity of the chalazal layer or inhibits a deleterious effect of the maternal tissue. The miniature endosperm, on the other hand, may lack this substance either partially or entirely, or may produce a different substance which promotes dissolution of the chalazal layer. In either case the breakdown of the connecting cells, and the resulting gap in the nutrient supply between ovary and endosperm causes miniature seed.

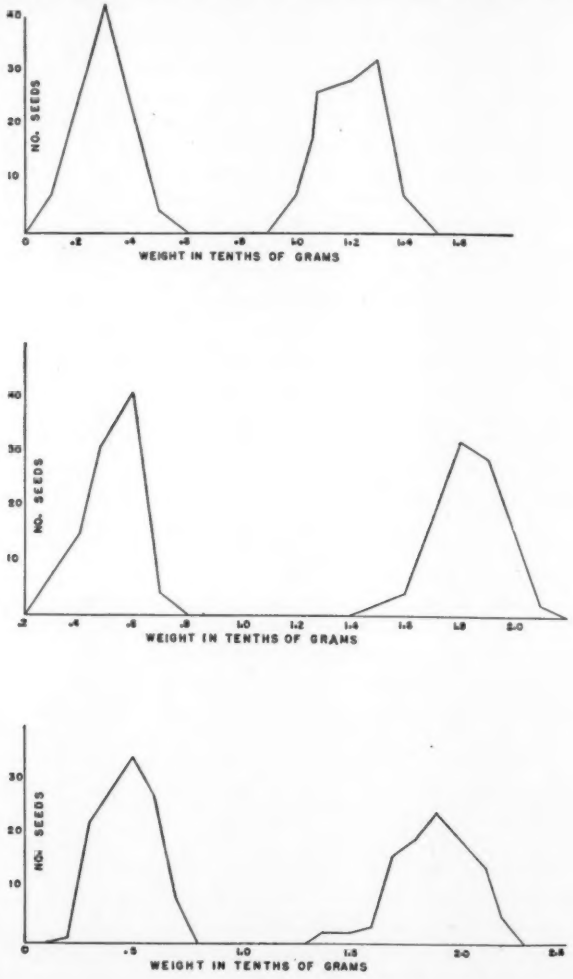






FIGURES 4–6.—Frequency graphs of weight of each kernel plotted against the number of kernels in each weight class for ears segregating miniature and normal, harvested at one week (fig. 4 above), two weeks (fig. 5 center), and three weeks (fig. 6 below).

These histological investigations correlate well with frequency graphs made by plotting the weight of each kernel against the number of kernels in each weight class for ears segregating miniature and normal seeds harvested at stated intervals from pollination to maturity (fig. 4–9). The first graph shows a unimodal curve typical of a rather homogeneous weight population. However, the graph representing the kernels taken from an ear harvested 14 days



FIGURES 7–9.—Frequency graphs of weight of each kernel plotted against the number of kernels in each weight class for ears segregating miniature and normal, harvested at four weeks (fig. 7 above), five weeks (fig. 8 center), and six weeks (fig. 9 below).

after pollination shows a distinct bimodal phase in the weight population. This bimodality is enhanced with time as the kernels develop into the normal, and into the thin, papery, miniature seeds.

SUMMARY

Miniature seed is unique among defective seed characters in that it combines a high degree of defectiveness (the weight is only one-fifth that of normal seeds from the same ear) with normal growth in both the gametophytic and sporophytic generations. The character is recessive to normal, epistatic to sugary and brittle, and is a single factor difference. The locus of the gene is not known. The miniature endosperm apparently is lacking a substance or substances found in normal endosperm which are necessary to continuity of the chalazal layer, or else it produces a substance which is deleterious to the connecting chalazal cells, for they begin to break down about nine days after pollination. Up to this point growth and development are apparently normal. After this point growth slows down and stops almost entirely by 14 days, whereas development continues. Embryo and endosperm are present in the same ratio as in normal kernels. An aleurone layer and corneous starch are present. The discontinuity of the chalazal layer and resulting interruption in nutrient supply to endosperm and embryo are responsible for the defective condition.

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WENTZ, J. B., 1924 Heritable characters of maize. XVIII. Miniature germ. J. Hered. **15**: 269–272.

PAVAN, C., *Chromosomal variation in Drosophila nebulosa.*—Received May 24, 1946.—The gene arrangement in the chromosomes of eight strains of *D. nebulosa* coming from Texas, from equatorial Brazil, and from southern Brazil, has been examined. The species has three pairs of chromosomes: a V-shaped X chromosome, a V-shaped and a rod-like autosome. In the salivary gland cells five long euchromatic strands are found. The gene arrangement in the V-shaped autosome has been found to be constant. That in the X chromosome is constant, except for a single inversion found in some individuals of one strain from Texas. One of the points of breakage in this inversion lies in the chromocentral heterochromatin. The gene arrangement in the rod-like autosome (the third chromosome) is very variable. Seventeen gene arrangements in this chromosome have been identified; they arise through recombination of eight separate inversions. Some groups of inversions are overlapping; this fact permits construction of schemes showing the probable descent relationships of the different gene arrangements. Among the inversions, some are geographically widely distributed while others have been found in only a single strain.

TAN, C. C., *Genetics of sexual isolation between Drosophila pseudoobscura and Drosophila persimilis.*—Received May 29, 1946.—Sexual preference tests were made, using different mutant strains of *Drosophila pseudoobscura*—namely, orange purple (*or pr*), orange (*or*), white (*w*), aristapedia (*ast*), Bare Curly (*Ba Cy*), yellow singed vermillion compressed short (*y sn v co sh*), and one wild type strain. The *y sn v co sh* males inseminate more females of their own kind than females of other mutant strains or of wild type. This effect depends upon the mutant yellow. Other tests showing preferential matings include: (1) wild type males inseminate more *or* than wild type females, (2) *or pr* males inseminate more *or pr* than wild type females, (3) *ast* males inseminate more *Ba Cy* than *ast* females, (4) *Ba Cy* males inseminate more *Ba Cy* than *ast* females, and (5) *or* males inseminate more *or* than *ast* females.

Hybrids of *D. pseudoobscura* and *D. persimilis* were obtained ranging in chromosomal constitution from individuals having all chromosomes of *D. pseudoobscura* to individuals having equal numbers of *D. pseudoobscura* and *D. persimilis* chromosomes. When representatives of the different classes of backcross hybrid females were placed together with *D. pseudoobscura* females and *D. pseudoobscura* males, slightly more hybrid than *D. pseudoobscura* females were inseminated.

When a mixture of backcross hybrid females and *D. persimilis* females are exposed to *D. persimilis* males, the results also show a slight preference for mating with the hybrids. But those classes of hybrid females which do not carry the X chromosome, or the second chromosome, of *D. persimilis* are preferred by *D. persimilis* males to a smaller extent than those females which carry these chromosomes.

When *D. persimilis* males are placed with *D. pseudoobscura* females and

with backcross hybrid females, the results show a significant preference for mating with the hybrids, with the exception of the four classes of hybrids which do not carry either an X chromosome or a second chromosome of *D. persimilis*. This suggests that the main factors which distinguish the mating behavior of *D. persimilis* from *D. pseudoobscura* lie in the X and the second chromosomes.

MEAD, S. W., P. W. GREGORY, and W. M. REGAN, *Deleterious recessive genes in dairy bulls selected at random.*—Received June 10, 1946.—The background of the University of California breeding experiment with dairy cattle is presented, with a description of the original stock. Six sires, five Jersey and one Holstein, were selected at random so far as recessive genes were concerned and were tested for recessives by sire-daughter matings and other mating tests. Although some of the recessive genes carried by the sires may perhaps have escaped detection, there is no doubt concerning the eight that were positively identified.

Two lethal genes were found—one conditioning imperfect epithelium, the other an achondroplasia-like anomaly. The nonlethal genes were those conditioning proportionate dwarfism, flexed pasterns, strabismus, and congenital cataract. Two different forms of female sterility that prevented heifers from conceiving or producing offspring were also encountered. In the sterility found, only in animals of the Jersey herd did all the affected heifers manifest normal heat periods. The sterility found only in the Holstein breed was associated with an abnormal estrous cycle, usually complete absence of heat. Both forms of sterility are sex-limited. There was no evidence that the fertility of heterozygous cows is impaired. One Jersey bull that was fully sterile was assumed to be homozygous for the Jersey type of female sterility. None of the sires proved were homozygous for the Holstein type of female sterility; but it is assumed that they are fertile. The expected proportions of sterility in heifers based upon the gene frequencies found are compared with the actual figures recorded in the literature.

The matings provided some data concerning the linkage relations of certain combinations of the mutant genes. The matings involving the combination of achondroplasia, strabismus, and flexed pasterns indicated that these genes are independent. Other matings indicated that cataract and the female sterility found in Jerseys are independent.

The feasibility of an organized program for amassing all kinds of genetic data on domestic animals is discussed.

MAMPELL, KLAUS, *Genic and nongenic transmission of mutator activity.*—Received June 15, 1946.—The genetic information concerning the mutator gene has been extended. Females are unable to induce primary or secondary mutations, because they do not produce the mutator substance. When they are given a Y chromosome, they can make and transmit the mutator substance, thus inducing primary and secondary mutations. Primary mutation is evidently increased in the male by a second Y chromosome.

Quantitative and qualitative variations in the mutability can be shown to be due to differential susceptibility of the genes to the mutator.

When the mutator is in cytoplasm (from the female) which has previously been influenced by the mutator substance, its effectiveness may be increased.

The mutations are genetic deficiencies, conceivably representing heterochromatin inactivations of genes rather than physical deficiencies.

High mutability of the same appearance as in the mutator strain has been observed in other strains; there, it is not due to the mutator gene. The possibility of transmission of the mutability by infection was tested.

Pairs of *melanogaster* were placed in cultures with adult *pseudoobscura* flies. Mutability is increased in the descendants of *melanogaster* raised in cultures with mutator or wild type males; it is not increased in cultures with mutator or wild type females. The mutability in infected strains tends thereafter to persist.

A hypothesis as to the nature and mode of action of the mutator particles and the mutator-plus particles has been presented. It has been argued that perhaps the normal symbiotic particles of one species may become parasitic virus particles when transferred to another species.

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